GENERAL ELECTRIC COMPANY
RESEARCH AND DEVELOPMENT PROGRAM
FOR THE DESTRUCTION OF PCBs

Sixth Progress Report

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Submitted by

Herman L. Finkbeiner and Stephen B. Hamilton

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

REGION I

MEMORANDUM

DATE: February 10, 1988

TO: Addressees

FROM: Frank Ciavattieri
New Bedford Harbor Project Manager

SUBJ: General Electric Progress Report, R & D Program for the Destruction of PCBs.

Enclosed is the latest progress report from General Electric's research and development program for the destruction of PCBs. This may provide some insight for their February 19th presentation at the Long Wharf Marriot, Boston. I look forward to seeing you there and please, bring your tough questions.

Addressees:		
	Allen Ikalainen	E.C. Jordan
	Dick McGrath	Battelle
	Don Phelps	Narragansett
	Dave Hansen	Narragansett
	Leroy Folmar	Narragansett
	Jim Lake	Narragansett
	Mark Otis	Corps-NED
	Danny Averett	Corps-WES
	Helen Waldorf	MA DEQE
	Judy Pederson	MA CZM
	Charles Bering	EPA
	Marcie Wetherbee	New Bedford
	Lydia VanHine	New Bedford
	Dr. Albert Robbat	Tufts University

CONTRIBUTORS

Donna L. Bedard, Lawrence H. Bopp, Michael J. Brennan, Ronald E. Brooks, John F. Brown, Jr., Marie L. Haberl, Frank J. Mondello, and Ronald Unterman

Biological Sciences Branch
General Electric Corporate Research and Development
Schenectady, New York

James C. Carnahan, Ralph J. May, and Robert E. Wagner

Organic and Polymer Characterization Unit
General Electric Corporate Research and Development
Schenectady, New York

Gary W. Yeager

Chemical Process Branch
General Electric Corporate Research and Development
Schenectady, New York

David P. Mobley, John B. McDermott, and Charles C. Schwartz

Chemical Engineering Branch
General Electric Corporate Research and Development
Schenectady, New York

Frank K. Higson, David T. Gibson, Louise M. Nadim, and Sadhana C. Singh

Center for Applied Microbiology
The University of Texas at Austin
Austin, Texas

James M. Tiedje, Stephen A. Boyd, and John F. Quensen

Department of Crop and Soil Sciences
Michigan State University
East Lansing, Michigan

Sandra L. Woods and Li Tang

Department of Civil Engineering
Oregon State University
Corvallis, Oregon

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

EXECUTIVE SUMMARY

Our research this year has focused on five major areas: (1) the biochemistry and genetics of bacterial oxidation of PCBs, (2) reductive dechlorination of PCBs by anaerobic communities, (3) analysis of environmental transformations of PCBs, (4) development of laboratory models for evaluating the biodegradation of PCBs on soil, and (5) development of laboratory models for evaluating surfactant extraction of PCBs from soil.

Biochemistry and genetics of bacterial oxidation of PCBs. Further studies of PCB congener specificity and metabolite production in *Pseudomonas putida* LB400 have been conducted both at GE and in Dr. Gibson's laboratory at the University of Texas at Austin. These studies have established that LB400 metabolizes PCBs in much the same way as *Alcaligenes eutrophus* H850. Both strains have the unusual ability to degrade certain congeners, such as 2,5,2',5'-CB, by a 3,4-dioxygenase attack, and both generate chloroacetophenones from the metabolism of PCBs containing a 3-, 2,5-, or 2,4,5-chlorophenyl ring. We have also demonstrated that both of these strains oxidize a 2-chlorophenyl ring of a PCB in preference to a 3- or 4-chlorophenyl ring. In contrast, most of the other strains we have studied are limited to the degradation of PCB congeners with open 2,3-sites, and most of these strains attack a 3- or 4-chlorophenyl ring in preference to a 2-chlorophenyl ring. Dr. Gibson's laboratory has also found evidence that LB400, and possibly H850, contain an esterase that catalyzes the methylation of the chlorobenzoic acids generated from PCB degradation.

We have determined that chloroacetophenones are produced as PCB metabolites in eight different strains of bacteria that differ considerably in congener specificity and in PCB-degradative competence. Chloroacetophenones are only produced from congeners containing a 3-chlorophenyl ring (or, in the case of H850 and LB400, a 2,5- or 2,4,5-chlorophenyl ring). We have proposed that these novel PCB metabolites might result from an alternative route of metabolism following 2,3-dioxygenase attack of a 3-chlorophenyl ring.

We expect to gain a clearer understanding of the biochemistry of PCB degradation through studies with isolated enzymes. In the past year we have made significant progress in purifying and characterizing enzymes of the biphenyl/PCB degradation pathway from cell-free extracts of LB400; 2,3-biphenyl catechol oxygenase has been purified and partially characterized, and 2-hydroxy-6-oxo-6-phenyl hexa-2,4-dienoic acid hydrolase (hydrase) and biphenyl/PCB dioxygenase have been partially purified. At this point a single protein fraction exhibits both 2,3- and 3,4-dioxygenase activity. However, because this fraction contains more than one protein it is not yet clear whether a single enzyme is responsible for both activities.

We have succeeded in cloning the genes encoding the first four enzymes of the biphenyl/PCB degradation pathway from LB400 and in expressing these genes in an

E. coli host. Several recombinant organisms contain all four enzymes; i.e., they degrade 2,3-CB to 2,3-dichlorobenzoic acid. These same recombinants also express the enzyme responsible for the degradation of 2,5,2',5'-CB, possibly a 3,4-dioxygenase. Subcloning experiments to identify the DNA fragments that contain the biphenyl/PCB degradation genes are currently in progress. These studies have already demonstrated that the genes that encode the first four enzymes of the biphenyl/PCB dioxygenase pathway are encoded on two DNA fragments sized at 6.6 and 2.9 kilobases.

Reductive dechlorination of PCBs in anaerobic communities. Drs. Tiedje, Quensen, and Boyd at Michigan State University have now observed anaerobic dechlorination of PCBs in laboratory experiments using batch cultures of Hudson River sediments. They reported that three of the five test PCB congeners added to the Hudson River sediments (2,3,4,5,6-, 2,3,4,6,2',4',5'-, and 2,4,3',4'-CB) were significantly depleted after one year, and they identified the dechlorination products of the first two. The most readily dechlorinated congener, 2,3,4,5,6-CB, was decreased by approximately 44%, and its dechlorination products, 2,3,4,6- and/or 2,3,5,6-CB (corresponding to 28% of the parent PCB), and possibly 2,4,6-CB were identified. Other observed changes that occurred in the congener profile of the background PCBs in the Hudson River sediments in the live samples, but not in the sterile controls, were interpreted as evidence of ongoing reductive dechlorination. These changes include significant increases in 2,4,2'-, 2,6,4'-, and 2,4,2',4'-CB, all of which have been previously identified as dechlorination products [Brown et al., 1987a].

Dr. Woods and her colleagues have been operating an anaerobic upflow sludge blanket reactor that was seeded with anaerobic Hudson River sediment from PCB "hot spots" and with a bacterial consortium that dechlorinates various chlorinated aromatic compounds. In this system PCBs are continuously added to the reactor and the effluent is monitored for the appearance of dechlorinated PCBs or other PCB metabolites. Throughout the past year there has been no evidence for the appearance of PCB metabolites. However, this does not preclude PCB degradation. The rapid dechlorination required in such a system depends on acclimation of a large population of PCB-dechlorinating organisms. Furthermore, if the rate of degradation of the metabolites is faster than that of the parent compound, detectable amounts of metabolites will accumulate only within a limited set of wastewater retention times. These studies are continuing using a submerged anaerobic filter reactor that should improve the retention of acclimated organisms in the system.

Environmental transformations of PCBs. Our analyses of PCB-contaminated sediments from the upper Acushnet River estuary (New Bedford, Massachusetts) and Escambia Bay (Pensacola, Florida), and of the PCBs in fish from New York Harbor have shown that reductive dechlorination occurs in marine as well as freshwater sediments. Furthermore, analyses of the freshwater sediments of the Housatonic River system have demonstrated that the Aroclor 1260 in Silver Lake (Pittsfield, Massachusetts) and Woods Pond (southwestern Massachusetts) is being

dechlorinated, but there is no evidence for dechlorination in the lightly contaminated Connecticut portions of the river. Thus far, we have seen evidence of reductive dechlorination in every heavily contaminated (PCB concentration >50 ppm) marine or freshwater sediment that we have examined. This work strengthens our contention that PCB dechlorination in anaerobic sediments is widespread in nature.

Laboratory models for evaluating the biodegradation of PCBs on soil. Our model studies indicate that PCB degradation on soils in situ is feasible, but that it will be significantly slower than in our conventional resting-cell assays. It will take weeks or months instead of hours or days to achieve the same degree of degradation in the field as that seen in the laboratory under optimal conditions (high cell concentration, optimal temperature and aeration), and repeated applications of bacteria will be necessary. Site preparation and development of mass culturing techniques for a site test at a drag strip contaminated with Aroclor 1242 have now been completed.

Process modeling for surfactant extraction of PCBs from soil. Our modeling studies have determined that an anionic surfactant (such as Surco 233) is preferable to a nonionic surfactant (such as Triton X-100) for use on the subfill soil at the Oakland site because the anionic surfactants do not adsorb to the clay, whereas the nonionic surfactants do. Stage calculations have demonstrated that soil contaminated with 1000 ppm of Aroclor 1260 can be cleaned to less than 10 ppm PCB in relatively few equilibrium stages.

Because of the fine particulate nature of clay, separation of the surfactant-PCB solution from the soil is difficult, but possible with the use of either large-area thickeners or centrifuges. We are also investigating methods for removing the PCB from the surfactant wash. Ideally we would like to remove the PCB and recycle the surfactant solution. However, none of the available options (adsorbents, liquid-liquid extraction, biodegradation) is satisfactory at present. The alternative, removal of both surfactant and PCB, looks more promising at this time. At GE we are currently investigating the use of divalent ions such as calcium to precipitate PCB-laden surfactant.

At Oregon State University, Dr. Woods and her colleagues are investigating the removal of PCBs from aqueous solutions by solvent sublation. This process involves the transfer of PCBs from the aqueous phase to a solvent phase by gas bubbles. In the past year Dr. Woods and her colleagues have studied the feasibility of using this process to separate PCBs from the aqueous surfactant waste that would result from surfactant extraction of PCBs. Preliminary studies conducted with an aqueous solution of 2,4,5-CB indicate that sublation is a promising method of PCB removal.

Chapter 1

OXIDATION OF SELECTED PCB CONGENERS BY *PSEUDOMONAS PUTIDA* LB400 AND *ALCALIGENES EUTROPHUS* H850

Frank K. Higson, David T. Gibson, Louise M. Nadim, and Sadhana C. Singh
Center for Applied Microbiology
The University of Texas at Austin
Austin, Texas

INTRODUCTION

Last year's report dealt largely with the degradation of 2,5,2′,5′-CB by *Alcaligenes eutrophus* H850. In that work we reported the novel oxidation of the tetrachlorobiphenyl at the 3,4 positions to form two *cis* dihydrodiol metabolites. This year we have investigated the degradation of 2,5,2′,5′-CB and other congeners — 2,5,2′-, 2,2′-, 4,4′-, 2,4,4′-, 2,4,3′,4′-, and 2,4,5,2′,5′-CB — by two strains selected for their wide-ranging degradative competence: H850 and *Pseudomonas putida* LB400. Study of the relative ease of degradation of the chosen congeners has shown the importance of the degree and position of chlorine substitution on the biphenyl nucleus when these congeners are incubated with either organism.

Studies have also been conducted on the degradation of 3,4-dihydroxybiphenyl by H850. This substrate was of interest because it was anticipated that the results obtained would provide information relating to the degradation of 2,5,2′,5′-CB.

RESULTS AND DISCUSSION

Metabolism of Selected PCBs

No substrate depletion and no metabolites were detected when biphenyl-grown or succinate/biphenyl-grown cells of LB400 or H850 were incubated with 4,4′-CB, or when succinate/biphenyl-grown cells of LB400 were incubated with 2,4,3′,4′-CB. Slight degradation of 2,4,4′-CB was observed when incubated with succinate/biphenyl-grown cultures of LB400 or of a mutant derived from it, FM408. The latter accumulates the 2,3-*cis*-dihydrodiol from biphenyl, but in the case of 2,4,4′-CB, no dihydrodiol was detected and small amounts of a metabolite were seen. Using GC-MS we have tentatively identified this metabolite as the methyl ester of chlorobenzoate.

LB400 catalyzed the rapid degradation of 2,2′-CB. Products were tentatively identified as a dihydrodiol and phenols formed by subsequent dehydration of the dihydrodiol. The methyl ester of 2-CBA was also detected. FM408 again failed to produce the dihydrodiol from 2,2′-CB, but did produce the methyl ester of 2-CBA.

Induced cells of LB400 rapidly oxidized 2,5,2′-CB to a dihydrodiol. Additional products were 2,5-CBA and its methyl ester. Cells of LB400 and H850, grown on succinate and biphenyl, both oxidized 2,5,2′,5′-CB to the 3,4- and 3,4,3′,4′-dihydrodiols described in last year's report. In addition, LB400 also formed trace amounts of 2′,5′-dichloroacetophenone (2′,5′-CA) and a compound tentatively identified from its mass spectrum as 1-(2,5-dichlorophenyl)ethanol. The same dihydrodiols were formed when 2,5,2′,5′-CB was incubated with FM408.

The congener 2,4,5,2′,5′-CB was degraded by succinate/biphenyl-grown cells of LB400 at a similar rate to 2.5,2′,5′-CB; a dihydrodiol was detected by GC-MS, consistent with oxidation at the 3,4-position of the 2,5 substituted ring. In the same analysis, 2,5- and 2,4,5-CA were both recognized as minor metabolites, which would indicate that this strain can oxidize the pentachlorobiphenyl at both aromatic rings.

Oxidation of 3,4-Dihydroxybiphenyl (3,4-DHB)

Succinate/biphenyl-grown cells of H850 and LB400 rapidly oxidized 3,4-DHB to a metabolite identified as the methyl ester of 3,4-dihydroxybenzoic acid by comparison of UV absorption, HPLC and GC retention time, and mass fragmentation pattern of the metabolite with a sample of the synthetic ester.

Neutral extracts also contained a compound that gave a molecular ion at *m/z* 220. This is the expected molecular ion for a dihydrodiol derivative of 3,4-dihydroxybiphenyl. Subsequent analyses of the trimethylsilyl (TMS) derivatives formed from compounds in the neutral extract provided further evidence for the formation of a compound that has been tentatively identified as 2,3-dihydro-2,3,3′,4′-tetrahydroxy biphenyl. A mutant strain of H850 (FM803) oxidized 3,4-DHB to significant amounts of the dihydrodiol. Studies are currently directed toward elucidation of the structure of this metabolite.

Mechanism of Methyl Ester Formation

Induced cells of LB400 oxidized 2,2′-, 2,4,4′-, and 2,5,2′-CB to the methyl esters of 2-, 4-, and 2,5-CBA, respectively. Methanol was used as a solvent for the PCBs in these experiments; consequently, 2,2′-CB was dissolved in deuterated methanol (CD_3OD) and the heavier molecular ion for the ester on GC-MS analysis indicated that the methyl group originated from methanol. Interestingly, LB400 also metabolizes 2,2′-CB added in N, N-dimethylformamide to methyl-2-CBA.

When CD_3OD was used as a solvent for 3,4-DHB the deuterated ester of 3,4-dihydroxybenzoic acid was formed. Control experiments have shown that the esterification is enzymatic. Moreover, when the sodium salts of 2-CBA and 3,4-dihydroxybenzoate were incubated with biphenyl-induced cells of either LB400 or H850, no ester formation could be detected.

SUMMARY AND CONCLUSIONS

1. The initial reaction in the degradation of biphenyl by bacteria involves dioxygenation at the 2,3-positions. However, chlorine substitution at the 4,4'-positions appears to inhibit degradation even if the substrate has 2,3-positions available for oxygenation. Substrates tested at concentrations of 45 μM were 4,4'-, 2,4,4'-, and 2,4,3',4'-CB. It should be noted that relative to 4,4'- and 2,4,3',4'-CB higher degradation of 2,4,4'-CB was observed. In this case *ortho*-chlorine substitution appears to enhance degradation, although it has been reported that degradation diminishes with increasing chlorine substitution (Furukawa et al., 1978 and 1979).

   In contrast, 2,2'-CB was rapidly metabolized: at a level of 45 μM, none remains after 1 h. Increased chlorine substitution on 2,2'-CB retarded degradation, consistent with the observations of Bedard and her colleagues (Bedard et al., 1986 and 1987a; Bopp, 1986) though the presence of 2,5-substitution seemed to countervail this effect somewhat.

2. The tetra- and pentachlorobiphenyls (2,5,2',5'- and 2,4,5,2',5'-CB) were degraded completely by LB400, though both lacked the 2,3-sites utilized by the biphenyl dioxygenase. The observation of ring-chlorinated acetophenones suggests that the 3,4-dihydrodiol of 2,5,2',5'-CB, previously believed only to form the *bis* diol, may be subject to dehydrogenation and ring cleavage to a limited extent.

3. An esterase may be present in LB400 that utilizes not only solvent methanol but also endogenous methyl donors in the formation of methyl esters of chlorobenzoates when certain PCBs are metabolized. Diversion of the degradation pathway to these products may prevent mineralization of the substrates.

FUTURE WORK

1. We will investigate the possible presence of two enzymes, one specific for biphenyl and the second for a PCB with no free 2,3-sites, in LB400. This is best approached by identification of the dioxygenase(s) in extracts of biphenyl-induced cells supplemented with cofactors.

2. The nature of induction of the enzymes responsible for biphenyl and 2,5,2',5'-CB degradation will be studied using chloramphenicol as a protein synthesis inhibitor. These investigations should permit us to assess the extent of constitutivity for these two substrates, a study that may provide evidence for two enzymes.

3. The methylase activity will be characterized in crude cell extracts from H850 and LB400, applying substituted benzoic acids as substrates.

4. Extensive use of the mutants of LB400 will be made with the view to identifying the intermediates formed from each congener. Application of FM408,

unable to metabolize biphenyl dihydrodiol, has already shown differences between PCB and biphenyl oxidation. Use of the mutants accumulating 2,3-DHB from biphenyl (FM903, 905) may show dihydroxy-derivatives of PCBs not hitherto identified in wild-type extracts.

Chapter 2

BIOCHEMISTRY OF PCB DEGRADATION IN BACTERIA: CONGENER SELECTIVITY AND DEGRADATIVE PRODUCTS

Donna L. Bedard and Marie L. Haberl
Biological Sciences Branch

Ralph J. May
Organic and Polymer Characterization Unit

Gary W. Yeager
Chemical Process Branch

General Electric Corporate Research and Development
Schenectady, New York

INTRODUCTION

Last year we reported that in *Alcaligenes eutrophus* H850, PCB congeners containing a 3-, 2,5-, or 2,4,5-chlorophenyl ring are oxidized through novel metabolites: ring-chlorinated acetophenones. We later learned that these transient intermediates are also produced from the metabolism of PCBs by *Pseudomonas putida*, LB400. Both of these strains degrade a wide variety of PCB congeners, including those with blocked 2,3-sites (such as 2,5,2′,5′-CB) and those with no adjacent unchlorinated carbons (such as 2,4,5,2′,4′,5′-CB). Furthermore, both strains degrade 2,5,2′,5′-CB (and presumably other congeners containing a 2,5-chlorophenyl ring) via dioxygenase attack at position 3,4 [GE reports 1985 and 1986, and Chapter 1].

Because the novel chloroacetophenone metabolites are produced from attack on a 2,5-chlorophenyl ring, we initially postulated that they might be formed from a novel pathway of PCB degradation initiated by a 3,4-dioxygenase. If this were true, these metabolites would not be found in bacteria that degrade PCBs exclusively by a 2,3-dioxygenase. However, if chloroacetophenones are products of a pathway initiated by a 2,3-dioxygenase, they should appear as metabolites of PCB degradation in a wide variety of PCB-degrading bacteria.

Therefore, one goal of our research this year was to determine whether chloroacetophenones are PCB metabolites in bacteria that degrade PCBs by a 2,3-dioxygenase. We were also interested in determining how much PCB is metabolized through acetophenones, and how chloroacetophenones are metabolized. Finally, we have furthered our studies of congener selectivity in various PCB-degrading bacteria in order to gain a better understanding of the structural factors that influence biodegradability.

RESULTS AND DISCUSSION

We studied the metabolism of eight di- and trichlorobiphenyls in eight strains of PCB-degrading bacteria. The bacteria include all seven species that we have isolated: *Alcaligenes eutrophus* H850, *Pseudomonas putida* LB400, *Corynebacterium* sp. MB1, *Alcaligenes faecalis* Pi434, *Pseudomonas cepacia* H201, *Pseudomonas testosteroni* H336 and H430, and *Pseudomonas* sp. (*Acidovorans* group) strain H1130. These strains vary considerably in congener selectivity and PCB-degradative activity (GE Report, 1985; Bedard et al., 1986). The PCB congeners studied were 2,3-, 2,3′-, 2,4′-, 3,3′-, 2,3,3′-, 2,5,3′-, 2,4,4′-, and 3,4,2′-CB. All experiments were conducted as previously described [GE Reports 1984, 1985, 1986] using resting cells that had been grown on biphenyl. Further details are given in the table footnotes.

Congener Selectivity and PCB Metabolism

Table 2-1 compares the maximum degradation in 24 h of each congener (250 μM) by each of the eight strains. As expected, all strains efficiently degraded 2,3-CB, which has an unchlorinated ring, and showed good degradation of 2,3′-, 2,4′-, and 2,3,3′-CB. Unexpectedly, however, all strains except MB1 exhibited very poor degradation of 3,3′-CB, and all strains except H850, LB400, and MB1 showed poor degradation of 2,5,3′- and 3,4,2′-CB. Equally surprising was the finding that H1130, H430, H201, and Pi434 all degraded 2,4,4′-CB to a greater extent than 2,4′-CB.

Table 2-1

PCB CONGENER SELECTIVITY OF EIGHT BACTERIAL STRAINS

PCB CONGENER	H1130	H430	Pi434	H201	MB1	H336	H850	LB400
2,3	●	●	●	●	●	●	●	●
2,3′	●	●	●	●	●	●	●	●
3,3′				•	●		•	•
2,3,3′	●	•	•	●	●	•	●	●
2,5,3′	•	•	•	•	•	•	●	●
2,4′	•	•	•	●	•	●	●	●
2,4,4′	●	●	●	●	●	●	•	●
3,4,2′	•	•	•	•	•	•	●	●

KEY: PERCENT DEGRADATION

● 80-100 • 20-39

● 60-79 • <20

● 40-59

Some of these results can be better understood by an analysis of the metabolic products. In particular, identification of the chlorobenzoic acids and, where present, chloroacetophenones generated from the oxidation of a PCB congener indicates which ring was attacked. H850 and LB400 metabolized 2,3'-, 2,4'-, and 3,4,2'-CB to 3-chlorobenzoic acid (3-CBA), 4-CBA, and 3,4-CBA, respectively. This indicates that these strains oxidize the 2-chlorophenyl ring in preference to a 3- or 4-chlorophenyl ring. H336 and Pi434 attacked both rings of 2,3'- and 2,4'-CB, but the four remaining bacterial strains apparently oxidized the 3- or 4-chlorophenyl ring of these congeners in preference to the 2-chlorophenyl ring. This may explain why the latter strains were unable to degrade 3,4,2'-CB.

In those cases where the 2-chlorophenyl ring was oxidized, the degraded PCB was almost completely metabolized to chlorobenzoic acid. This was not the case for the oxidation of a 3- or 4-chlorophenyl ring. In strains that attacked the 4-chlorophenyl ring of 2,4'- and 2,4,4'-CB, the metabolism of these congeners was accompanied by the appearance of a bright yellow color, indicative of accumulation of the ring-fission product. The accumulation of ring-fission product in PCB congeners chlorinated at positions 2,4' has previously been noted [Furukawa et al., 1978]. In fact, a chlorine at carbon 4 of the ring-fission product would be expected to stabilize this compound. Furthermore, the *ortho* chlorine on the unattacked ring might sterically impair access of the hydrase that catalyzes the cleavage necessary to form chlorobenzoic acid (see Figure 3-1).

All strains except H850 and LB400 oxidized the 3-chlorophenyl ring of 2,3'-CB, and all eight strains attacked the 3-chlorophenyl ring of 2,3,3'-CB. However, the products, chlorobenzoic acid and chloroacetophenone (see Table 2-2), represented only 30-50% of the degraded PCB. We reported last year [GE Report, 1986] that the chloroacetophenones are transient intermediates that are further metabolized in strain H850. Perhaps this is also true in the other PCB-degrading strains.

The differences in biodegradability of 2,3'-, 2,3,3'-, 3,3'-, and 2,5,3'-CB are more difficult to explain. Table 2-3 shows the structure of these congeners and the maximum degradation by each strain. The poor degradation of 3,3'-CB by all strains is striking, particularly since 2,3,3'-CB, which has chlorines at the same positions plus an additional *ortho* chlorine, was degraded to a much greater extent. It would appear that in this case the *ortho* chlorine on the unattacked ring enhances biodegradation. However, the data for 2,5,3'-CB clearly demonstrate that the addition of an *ortho* chlorine is not sufficient; the *ortho* chlorine must be adjacent to the *meta* chlorine to enhance biodegradation. It is also apparent from these data that MB1 differs from the other 2,3-dioxygenase-type strains in its superior ability to degrade 3,3'-CB and 2,5,3'-CB, although it is not clear why.

The higher degradation of 2,5,3'-CB relative to 3,3'- and 2,3,3'-CB by strains H850 and LB400 suggests that these strains may preferentially degrade 2,5,3'-CB by 3,4-dioxygenase attack on the 2,5-chlorophenyl ring rather than by 2,3-dioxygenase attack of the 3-chlorophenyl ring. This hypothesis is supported by two observations: (1) Only very small amounts (corresponding to less than 1% of the de-

Table 2-2

DEGRADATION PRODUCTS OF 2,3,3′-CB

Strain	2,3,3′-CB Degraded (nmol)	Percent of PCB recovered as products		
		2′3′-CA	2,3-CBA	Total CA and CBA
H1130	158	23	13	36
H430	80	15	38	53
H201	125	12	24	36
MB1	123	25	9	34
Pi434	85	16	35-40	51-56
H850	123	9	24	33
LB400	163	14	11	25
H336	55	24	23	47

The concentration of 2,3,3′-CB was 250 μM (250 nmol/ml). Biphenyl-grown cells were washed and incubated with PCB at 30 °C for 24 h.

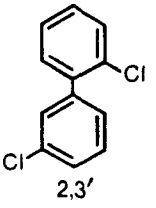
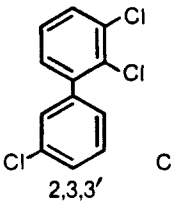
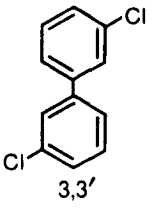
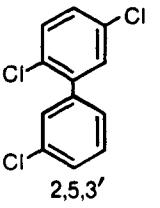
graded PCB) of 2,5-CBA and 2′,5′-CA are generated from the degradation of 2,5,3′-CB by H850 and LB400. In contrast, for MB1 these products correspond to 18% of the degraded PCB. (2) Both H850 and LB400 accumulate a metabolite of 2,5,3′-CB that has been tentatively identified as a dihydro-dihydroxy-trichlorobiphenyl, the metabolite that would be expected from a 3,4-dioxygenase attack on the 2,5-chlorophenyl ring. This metabolite was not seen in MB1.

Chloroacetophenones as PCB Metabolites

None of the bacterial strains tested generated chloroacetophenones from attack on an unchlorinated ring or a 2- or 4-chlorophenyl ring, but all eight strains produced chloroacetophenone as well as chlorobenzoic acid from oxidation of the 3-chlorophenyl ring of 2,3′- and 2,3,3′-CB. The data shown in Table 2-2 show that in all strains the 2′,3′-CA represented a significant amount of the metabolic products recovered from the degradation of 2,3,3′-CB. Because there is no evidence of 3,4-dioxygenase activity in any of the strains except H850 and LB400, these findings suggest that the chloroacetophenones are alternative products of a 2,3-dioxygenase pathway. We have proposed a pathway for the formation of both chloroacetophenones and chlorobenzoic acids following 2,3-dioxygenase attack at

Table 2-3

INFLUENCE OF THE CHLORINATION PATTERN OF THE UNATTACKED RING ON THE DEGRADATION OF PCBs

BACTERIAL STRAIN	PCB DEGRADED (nmol/ml/24h)			
	 2,3′	 2,3,3′	 3,3′	 2,5,3′
H1130	155	158	0	<25
H430	165	80	0	<25
H201	195	125	40	<25
MB1	170	123	98	90
Pi434	145	85	0	30
H850	*	123	32	220
LB400	*	163	<25	180
H336	*	55	0	<25

*Data not included because in these strains a considerable amount of degradation is due to attack on the 2-chlorophenyl ring

carbons 5,6 of the 3-chlorophenyl ring (Figure 2-1). As shown in the proposed pathway, the addition of H_2O and the subsequent loss of HCl would result in the loss of the *meta* chlorine at carbon 3 and its replacement by a hydroxyl group. Subsequent cleavage between carbons 2 and 3 initiated by hydroxyl ion attack at carbon 3 of the keto-form of this compound would generate chloroacetophenone, while cleavage between carbons 1 and 2 initiated by hydroxyl ion attack at carbon 1 would yield chlorobenzoic acid.

The proposed pathway explains why chloroacetophenones result from attack on a 3-chlorophenyl ring but not a 2- or 4-chlorophenyl ring. However, in H850 and LB400 chloroacetophenones are also generated from attack on a 2,5- or a 2,4,5-chlorophenyl ring. These might result from a novel route of metabolism initiated by 3,4-dioxygenase attack (or possibly, in the case of the 2,4,5-chlorophenyl ring, from monooxygenase attack). However, it is also possible that the chloroacetophenone produced from oxidation of 2,5- and 2,4,5-chlorophenyl rings results from a 2,3-dioxygenase attack, even though this requires attack at a chlorinated position (the chlorine at position 2). The latter possibility would be consistent with the fact

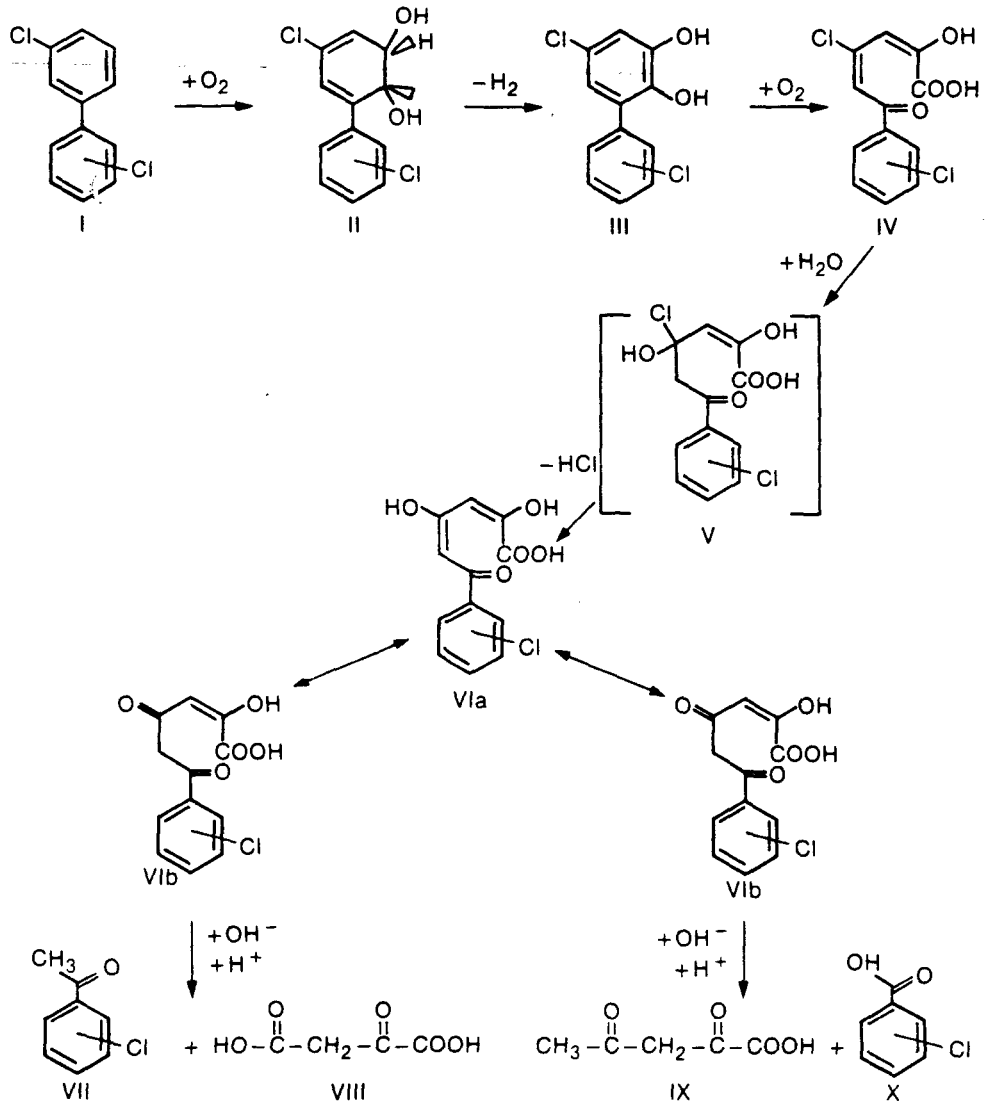


Figure 2-1. **Proposed pathway for degradation of PCBs via 2,3-dioxygenase attack on a 3-chlorophenyl ring. The compounds shown are (I) a chlorobiphenyl containing a 3-chlorophenyl ring, (II) 2,3-dihydro-2,3-dihydroxy-5-chlorobiphenyl, (III) 2,3-dihydroxy-5-chlorobiphenyl, (IV) 2-hydroxy-4-chloro-6-oxo-6-chlorophenyl hexa-2,4-dienoic acid (identical to the *meta* ring-fission product except for the *meta* chlorine), (V) 2,4-dihydroxy-4-chloro-6-oxo-6-chlorophenyl-2-hexenoic acid, (VIa) and (VIb) enol and keto forms of 2-hydroxy-4,6-dioxo-6-chlorophenyl-2-hexenoic acid, (VII) chloroacetophenone, (VIII) oxalacetic acid, (IX) acetylpyruvic acid, (X) chlorobenzoic acid.**

that in several instances a PCB congener with an additional *ortho* chlorine was more easily degraded by H850 than a congener with the same chlorine substitution pattern that lacked the *ortho* chlorine (Bedard et al., 1987b).

Metabolism of Chloroacetophenones

Last year we reported that chloroacetophenones were metabolized by H850. This is also the case for LB400. To investigate this metabolism we incubated 2',3'-CA (100 μM) with biphenyl-grown resting cells of each of these strains. Both strains completely metabolized the chloroacetophenone. In LB400 the chloroacetophenone was completely metabolized to a single metabolite that was identified by GC-MS and by coelution with an authentic standard as 1-(2,3-dichlorophenyl)-ethanol (2,3-DCPE). Because only a small amount of this product (representing ca. 3-6% of the metabolized chloroacetophenone) was seen in H850, it is not yet clear whether chloroacetophenone is metabolized through chlorophenyl ethanol or through some unidentified intermediate in this strain. None of the chloroacetophenone was metabolized to chlorobenzoic acid in either LB400 or H850, and no other metabolites were found.

SUMMARY

We have gained further insight into the bases of congener selectivity of PCB-degrading bacteria. The two organisms with the broadest PCB-degradative ability, H850 and LB400, attack a 2-chlorophenyl ring in preference to a 3- or 4-chlorophenyl ring, whereas the remaining six strains preferentially attacked a 3- or 4-chlorophenyl ring. Furthermore, we have found further evidence that the chlorine substitution on the unattacked ring may affect the biodegradability of PCB congener, hence 2,3'-CB and 2,3,3'-CB were both rapidly degraded by attack on the 3-chlorophenyl ring whereas 3,3'-CB and 2,5,3'-CB were not.

We have determined that chloroacetophenones are PCB metabolites in eight different strains of PCB-degrading bacteria that differ considerably in PCB-degradative competence. We have proposed that these novel metabolites result from an alternate route of metabolism of a 3-chlorophenyl ring following a 2,3-dioxygenase attack. The chloroacetophenones are transient intermediates. In strain LB400 the chloroacetophenone was completely metabolized to chlorophenyl ethanol; in strain H850 the chloroacetophenone was completely metabolized but no final product was identified.

FUTURE PLANS

In the coming year we plan to study PCB metabolism in mutant strains blocked at various steps of the 2,3-dioxygenase pathway (See Figure 3-1). In particular, we will characterize the congener selectivity pattern of a mutant strain of LB400 that is unable to oxidize biphenyl; i.e., it is a dioxygenase mutant. In addition, we will study PCB metabolism in mutant strains of H850 and/or LB400 that accumulate the

ring-fission product as the result of a mutation in the hydrase that catalyzes the formation of chlorobenzoic acid. One objective of this work will be to determine if PCBs are metabolized by alternative pathways when the 2,3-dioxygenase pathway is blocked.

We also intend to investigate the possibility of using aerobic bacteria to dechlorinate PCBs. We have recently demonstrated that dechlorinated Aroclor 1242 generated by a *meta-, para*-dechlorinating agent is easily oxidized by H850 [Bedard et al.,1987b], and we anticipate that the same would be true for dechlorinated Aroclor 1254 or 1260. Bacterial hydrogenases are enzymes that enable some bacteria to grow autotrophically on CO_2, O_2, and H_2. These enzymes contain nickel at the active site and are able to reduce a broad range of substrates [Cammack and Yates, 1986]. They are good candidates for aerobic PCB dechlorinases because it has been shown that hydrided (Raney) nickel and nickel-hydrogen combinations can catalyze the hydrodechlorination of PCBs [LaPierre et al., 1978; Dennis et al., 1979]. To investigate the potential use of hydrogenases as dechlorinating agents we will use H16, a strain of *Alcaligenes eutrophus* (the same species as H850) that contains two different hydrogenases [Schneider and Schlegel, 1976; Schink and Schlegel, 1979 and 1980]. Both hydrogenases have been well-characterized biochemically, and both are encoded on a transmissible plasmid that could be mobilized into a PCB-oxidizing strain such as H850.

Chapter 3

CELL-FREE SYSTEMS AND PCB BIOCHEMISTRY

Lawrence H. Bopp
Biological Sciences Branch
General Electric Corporate Research and Development
Schenectady, New York

INTRODUCTION

Both *Pseudomonas putida* LB400 and *Alcaligenes eutrophus* H850 have the ability to degrade PCBs in unusual ways. For example, they can degrade 2,5,2',5'-CB via a 3,4-dioxgenase attack to yield *cis*-3,4-dihydroxy-3,4-dihydro-2,5,2',5'-tetrachlorobiphenyl [GE Reports 1985, 1986; Nadim et al., in press]. In addition, they can degrade PCBs lacking vicinal hydrogen atoms (e.g., 2,4,5,2',4',5'-CB) [Bopp, 1986; Bedard et al., 1987b], and they produce chloroacetophenones as metabolites of some PCBs [Chapters 1 and 2; GE Reports 1985, 1986; Bedard et al., 1987a]. Both of these organisms are also able to degrade several PCB congeners via the more conventional 2,3-dioxygenase pathway (Figure 3-1). As our understanding of the complexity of PCB catabolism in these organisms has increased, it has become evident that less ambiguous methods than the whole cell studies used to date will be required in order to understand this complex biochemistry.

Two approaches have been developed. The first is a genetic approach (described in Chapter 4), in which the techniques of modern molecular biology are being applied to isolate and characterize the genes responsible for the unusual biochemistry described above. The second is a biochemical approach. We have developed cell-free preparations from LB400 that contain many of the enzymes of interest. These crude extracts are being fractionated and the enzymes of interest purified and characterized. By removing individual enzymes from the cells, we hope to gain a clearer understanding of their kinetics and substrate specificities.

RESULTS AND DISCUSSION

2,3-Biphenyl Catechol Oxygenase (BPCO)

A cell-free extract was prepared from biphenyl-grown LB400 cells and fractionated by differential centrifugation, as described previously [GE Report, 1986]. The membrane-free fraction (110,000 × g supernatant) was then further fractionated by fast protein liquid chromatography (FPLC) on an anion exchange column. Most of the BPCO activity was present in a single fraction containing approximately 17% of the total protein present in the material applied to the column. However, this fraction also contained a small amount of the succeeding enzyme, the 2-hydroxy-6-oxo-phenylhexa-2,4-dienoic acid hydrolase (hydrase).

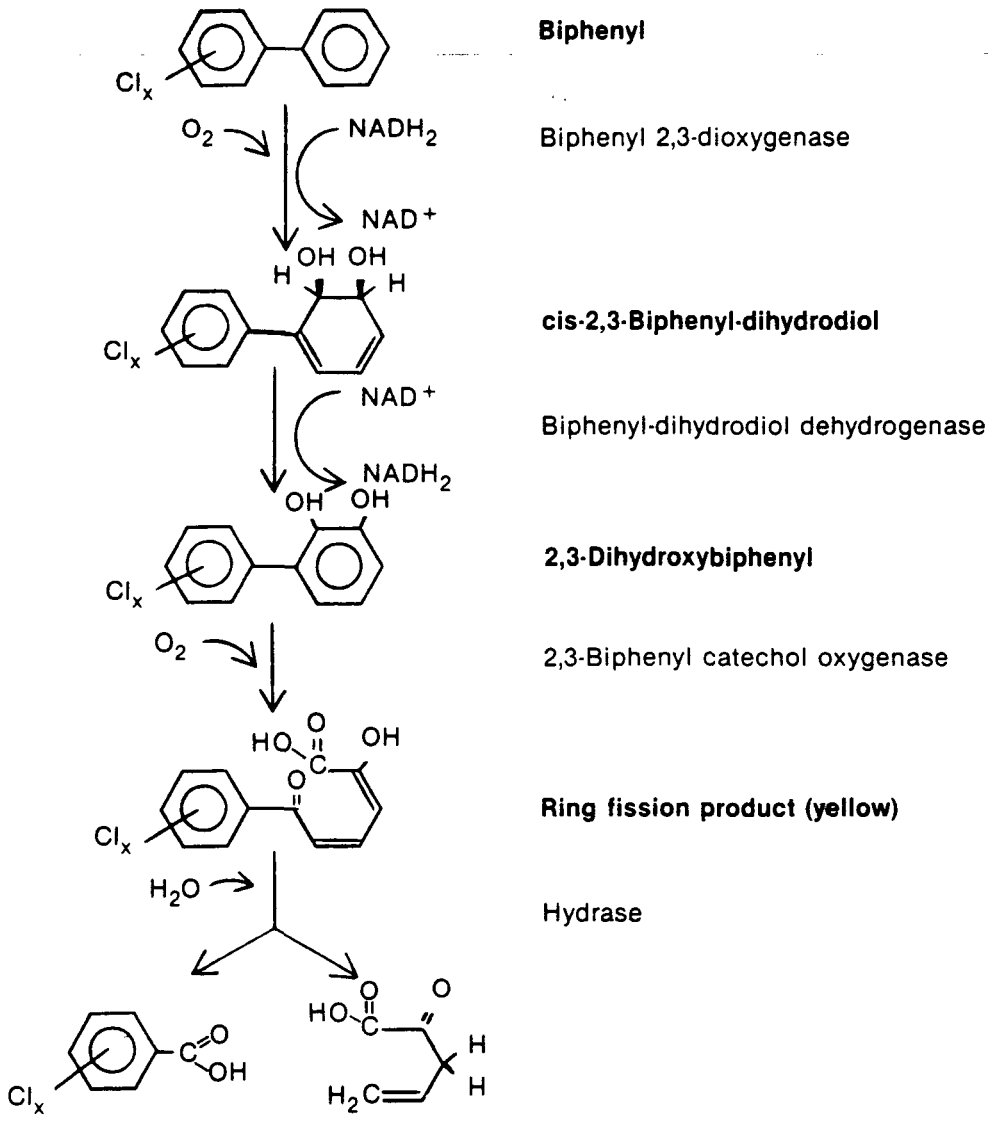


Figure 3-1. **Degradation of biphenyl and PCBs by the biphenyl 2,3-dioxygenase pathway.**

Further purification of BPCO was accomplished by FPLC using a gel filtration column. The fractions were analyzed for enzymatic activity by following either the appearance of product (λ_{max} = 434 nm) or the disappearance of substrate (λ_{max} = 249 and 289 nm). BPCO activity was usually found in a single fraction. This and neighboring fractions were analyzed by SDS polyacrylamide gel electrophoresis (PAGE). The results indicate that the active fraction contains one major protein with a molecular weight >200,000 daltons. Upon heating in SDS for 10 min at 100 °C, this large protein was no longer present, but was replaced by a single band with a molecular weight of approximately 32,000 daltons. Thus it appears that the BPCO enzyme consists of a multimer of 32,000-dalton subunits. The K_M of the enzyme after purification by gel filtration was 96 μM. The specific activity was 276 μmol/mg protein/min.

This enzyme bears some similarity to a BPCO isolated by Furukawa et al. [1987] from a PCB-degrading strain of *Pseudomonas pseudoalcaligenes*. However, there appear to be some fundamental differences between the enzyme described by Furukawa's group and that isolated from LB400. Their enzyme contains Fe(II) and has a strong absorbance at 412 nm. This iron is rapidly oxidized to Fe(III) in vitro and is readily lost during passage through a gel filtration column. In either case catalytic activity is lost. The BPCO from LB400 has no significant absorbance at 412 nm and loses little activity upon passage through a similar column. Furthermore, the catalytic activity of the isolated enzyme is stable for several hours at room temperature under oxidative conditions.

The gene for BPCO in LB400 has been cloned in *E. coli* and localized to a 2.0 kilobase (kb) piece of DNA [Chapter 4]. This fragment may also carry the gene for hydrase activity. Since a protein of 32,000 daltons requires a gene of approximately 835 base pairs (bp), it is reasonable to conclude that the 32,000-dalton protein characterized by PAGE is, in fact, the only peptide in the BPCO enzyme. This would mean that approximately 1.1 kb of the DNA fragment in question is available for another gene.

2-Hydroxy-6-oxo-6-phenyl Hexa-2,4-dienoic Acid Hydrolase (Hydrase)

The FPLC fraction containing the major hydrase activity was further fractionated by gel filtration as described above. The active form of the enzyme eluted from the column between molecular weight standards of 116,000 and 205,000 daltons. Fractions were screened for enzymatic activity using substrate that had been synthesized by *meta*-cleavage of 2,3-dihydroxybiphenyl using the purified BPCO enzyme. This material was extracted with ether following acidification and then neutralized and reverse extracted into water. Thus, a purified enzyme was used to synthesize the substrate for the next enzyme in the sequence. The hydrase has not been further characterized.

Chlorobiphenyl Dioxygenase

One of the major objectives of this line of investigation is to characterize the initial enzyme(s) in the degradation of PCBs. Evidence from whole-cell experiments with *P. putida* LB400 suggests that the 2,3-dioxygenase is inducible, while the 3,4-dioxygenase is constitutive [GE Report, 1985]. This phenomenon has also been observed in strain H850. If these observations are correct, one should be able to demonstrate the presence of both enzymes in cells grown on biphenyl (induced), but some factor necessary for 2,3-dioxygenase activity should be absent in cells grown on glucose (uninduced).

Initially, the 110,000 × g supernatant from biphenyl-grown LB400 was screened for activity against 2,3-, 2,4,4′-, and 2,5,2′,5′-CB. All three congeners were degraded: the 2,3-CB to 2,3-CBA and the others to as yet unidentified metabolites. The membrane fraction (110,000 × g pellet) contained no activity against these PCBs, and addition of the membrane fraction back to the soluble fraction did not enhance the activity. Thus, it was apparent that both 2,3- and 3,4-dioxygenase activities could be obtained in a cell-free system, and that the activity was soluble and not membrane-associated.

This membrane-free preparation was further fractioned by FPLC such that the chlorobiphenyl dioxygenase activities were both present in a single fraction. It was necessary to add NADH as a cofactor to obtain activity. 2,3-CB was degraded to the corresponding *cis*-2,3-dihydroxy-2,3-dihydrobiphenyl (*cis*-2,3-biphenyl-dihydrodiol). The metabolites of 2,4,4′- and 2,5,2′,5′-CB were not identified. Although the enzymatic activities of interest were still present and had been separated from the bulk of the protein present in the 110,000 × g supernatant, the two dioxygenase activities were still unresolved.

SUMMARY AND CONCLUSIONS

It is apparent from this work that it is possible to obtain enzymatic activity in the pathways of interest in a cell-free system. Furthermore, the results obtained so far show that purification of the enzymes involved in PCB degradation is feasible. The BPCO isolated from LB400 shows superficial similarities to that described by Furukawa et al. [1987], but appears different in that it is much more stable under assay conditions. The K_M, which reflects the affinity of the substrate for the enzyme, is similar, but the specific activity of the enzyme from LB400 is greater. This may be simply because the enzyme is more stable during the assay used to measure its activity.

The 2,3- and 3,4-dioxygenase activities have been found to be associated with soluble enzymes and have been partially purified but not separated from one another. Both activities require NADH as a cofactor. No bacteria other than H850 and LB400 has been shown to have 3,4-dioxygenase activity, so it is important to understand if and how this activity is related to other chlorobiphenyl dioxygenase activities in this and other organisms.

FUTURE PLANS

It is apparent from the work with BPCO that the biochemical and genetic approaches to understanding the complex pathways involved in PCB degradation are complementary. Preliminary results with the dioxygenase(s) also suggest that this coordinated approach will greatly facilitate understanding the biochemistry. The primary focus of the enzyme work will be to try to characterize the chlorobiphenyl dioxygenase activities of LB400, specifically addressing the question of how 2,3- and 3,4-dioxygenase activities are related. The work may be more fruitful if the genes for these activities can be separated and analyzed. The ultimate objective is to understand how the enzymes work and how they are regulated. In this way it may be possible to control or modify their activities to our advantage.

Chapter 4

MOLECULAR CLONING OF THE GENES FOR BACTERIAL PCB BIODEGRADATION

Frank J. Mondello
Biological Sciences Branch
General Electric Corporate Research and Development
Schenectady, New York

INTRODUCTION

The biodegradation of PCBs by *Pseudomonas putida* LB400 and *Alcaligenes eutrophus* H850 has been found to involve a complex series of enzymatic reactions that are difficult to study in the wild type strains. One of the problems is that these organisms may contain several degradative enzymes with overlapping substrate specificities. For example, the initial event in the pathway of PCB metabolism involves a dioxygenase reaction. At least two distinct types of dioxygenase activities (2,3- and 3,4-), have been identified in these organisms [GE Report, 1986]. We have not yet been able to determine if these activities are the result of one or more enzymes.

It is essential that we fully understand the initial dioxygenase reactions, as the enzyme(s) responsible for these activities are by far the most important in the pathway. This is because the congener specificity of the dioxygenase determines which PCBs are degraded, and its site of attack seems to guide the subsequent reaction steps [GE Report, 1986]. In addition, the level of dioxygenase activity sets the maximum rate of PCB metabolism. (No matter how active the other enzymes, PCBs cannot be degraded faster than they are initially oxidized).

One of our major objectives has been to isolate the genes encoding PCB/biphenyl degradation. This would greatly enhance our ability to study the specificity, structure, and expression, not only of the dioxygenase(s), but of all the enzymes in the pathway. Furthermore, using genetic engineering technology we may be able to manipulate these genes to enhance the PCB degradative competence of our organisms.

Genomic Libraries and Cosmid Cloning

In order to isolate the genes for PCB metabolism it was first necessary to generate "genomic libraries" of *P. putida* LB400 and *A. eutrophus* H850. Each of these libraries consists of a series of recombinant plasmids containing randomly generated fragments of LB400 or H850 DNA. By producing large numbers of recombinant strains, we can be assured of having cloned virtually all of the DNA sequences of the organisms. The host strain containing these recombinant plasmids

can then be screened for the ability to metabolize PCBs/biphenyl.

We have chosen to use "cosmid" cloning vectors for these experiments. A cosmid vector is a plasmid into which the *cos* site of the bacterial virus *Lambda* has been inserted [Collins and Hohn, 1978]. Such molecules, under certain conditions, can be packaged into bacteriophage *Lambda* capsids, and then used to infect *E. coli* host cells. There are two major advantages to these vectors. They increase the efficiency of introducing the recombinant molecules into the host strain, and allow for the cloning of unusually large DNA fragments (25 to 35 kilobases). (Cloning larger pieces of DNA increases the probability of isolating multiple PCB metabolism genes on the same fragment.)

PROCEDURES

Cloning vector pMMB34 is a 13.75 kilobase (kb) derivative of plasmid RSF1010 and contains the *cos* site as well as a gene for kanamycin resistance [Frey et al., 1983]. It is a wide-host-range plasmid and can be mobilized into a large number of bacterial genera. Purified pMMB34 DNA was linearized with a restriction enzyme and treated with alkaline phosphatase to prevent recircularization or polymerization of the vector. LB400 and H850 DNAs were purified and subsequently cut with restriction enzymes. DNA fragments of 25 to 35 kb were isolated using sucrose-gradient centrifugation. The sized DNA molecules from LB400 and H850 were separately mixed with and joined to the treated vector DNA under conditions that promote the formation of concatameric molecules. *Lambda* packaging extracts were used to insert these DNAs into empty virus particles. These pseudo-viruses were then used to inject the recombinant plasmids into cells of *E. coli* HB101. Cells receiving these plasmids were selected by their resistance to kanamycin.

RESULTS AND DISCUSSION

Construction of Genomic Libraries

Using the formula of Clarke and Carbon [1976], the number of recombinant strains required to achieve a genomic library can be calculated. Assuming genome sizes of 3,000 kb and an average fragment size of 30 kb, a gene bank representing 99% of the sequences in an organism would require approximately 500 recombinant strains. The numbers of such strains generated from LB400 and H850 DNA were approximately 3,500 and 35,000 respectively, and were thus in vast excess of that required for fully representative genomic libraries.

Screening for Enzymes of Biphenyl/PCB Metabolism

Approximately 1,600 recombinant *E. coli* strains containing LB400 DNA fragments and 6,000 that contain H850 sequences were tested for the presence of 2,3-biphenyl catechol oxygenase (BPCO) activity. This enzyme of the biphenyl/PCB pathway is responsible for the conversion of 2,3-dihydroxybiphenyl to a bright yellow metabolite [GE Report, 1986]. Thus far, five of the strains containing LB400

DNA have been found to express biphenyl catechol oxygenase. The recombinant plasmids responsible for mediating this activity have been designated as pGEM400, 410, 420, 430, and 440.

Using the rapid enzyme screening procedures described elsewhere, [GE Report, 1986], the strains containing pGEM400-440 were examined for the presence of additional biphenyl/PCB activities. As shown in Table 4-1, at least three of the pGEM plasmids have been found to encode the first four enzymes of biphenyl metabolism. As expected, none of these activities could be detected in strains containing the unmodified vector plasmid pMMB34.

Of the 6,000 H850 derived clones tested, none were found to have BPCO activity. This might indicate that the *E. coli* host strain is unable to express genes from *A. eutrophus*, or that the expression of these genes may be lethal to the cell.

Table 4-1

SCREENING FOR ENZYMES OF THE BIPHENYL PATHWAY

Plasmid	Enzymatic Activity Tested			
	Biphenyl Dioxygenase	Biphenyl Dihydrodiol Dehydrogenase	Biphenyl Catechol Oxygenase	Hydrase
pGEM400	+/−	+	+	+/−
pGEM410	+	+	+	+
pGEM420	+	+	+	+
pGEM430	−	−	+	+/−
pGEM440	+	+	+	+
pMMB34	−	−	−	−

PCB Metabolism by Recombinant Strains

Strains of *E. coli* containing the individual pGEM plasmids were tested for their ability to degrade several polychlorinated biphenyls in resting cell assays [GE Report, 1985]. Preliminary results indicate that the culture with the highest activity is that containing plasmid pGEM410. As shown in Table 4-2, this strain was able to degrade 35% of a 250-μM solution of 2,3-dichlorobiphenyl in 24 hours. Depletion of this PCB was accompanied by the appearance of a metabolite with a GC retention time identical to that of 2,3-dichlorobenzoic acid. This is important in that it demonstrates unequivocally the expression of at least the first four enzymes of the 2,3-dioxygenase pathway of PCB metabolism.

Also of great interest is the ability of this strain to degrade 2,5,2'5'-tetrachlorobiphenyl (Table 4-2). It is the ability of LB400 and H850 to metabolize this and similar congeners that sets them apart from other PCB-degrading strains.

Table 4-2

PCB METABOLISM BY RECOMBINANT AND WILD-TYPE STRAINS

PCB Congener	Concentration (μM)	% Depletion[a]		
		HB101 (pGEM410)	LB400	HB101 (pMMB34)
2,3	250	35	100	0
2,5,2',5'	20	17	100	0

[a] Incubation time 24 h

As yet, metabolite production from the oxidation of 2,5,2',5'-CB by the recombinant strain has not been detected. It should be noted, however, that the major metabolite of this congener produced by LB400 and H850 is a 3,4-dihyrodiol [GE Report, 1986]. Thus it might be speculated that the gene encoding the 3,4-dioxygenase activity may also be present on pGEM410. Similar results have been found for the strain containing plasmid pGEM420, although its level of activity is somewhat lower.

Subcloning of the Genes for PCB Metabolism

Subcloning experiments have been initiated in order to eliminate from pGEM410 the DNA sequences unrelated to the PCB degradative pathway. The digestion of pGEM410 with the restriction endonuclease *Eco*RI results in the formation of eight fragments with sizes of $\gtrsim$16, 6.6, 6.0, 2.9, 2.3, 2.1, 0.8, and 0.6 kb. To determine whether PCB/biphenyl metabolism is associated with one of these DNA fragments, purified pGEM410 DNA was cut with *Eco*RI, and the fragments randomly joined together and introduced into an *E. coli* host. Strains were isolated that demonstrated kanamycin resistance and BPCO activity. Plasmid DNA from these strains was then isolated and the *Eco*RI restriction fragment patterns examined.

In testing the plasmids from >50 subclones with BPCO activity, all were found to contain the 6.6-kb *Eco*RI fragment. This fragment does not occur in strains that do not have this activity. From this we conclude that the gene for BPCO activity is present on the 6.6-kb *Eco*RI fragment of pGEM410. Preliminary results indicate that in addition to BPCO, this fragment also encodes the genes for the dihydrodiol dehydrogenase and hydrase enzymes of the PCB/biphenyl pathway (Table 4-3).

At this time five subclones have been identified that express biphenyl dioxygenase activity. The plasmid DNA in each of these strains contains *Eco*RI fragments of 6.6 and 2.9 kb. Apparently both of these fragments are required for dioxygenase activity, because no activity has been detected in strains that contain only one of these fragments (Table 4-3).

Table 4-3

DNA FRAGMENTS ASSOCIATED WITH ENZYMATIC ACTIVITY

Enzyme Activity	Fragment sizes[a]		
	6.6	2.9 6.6	2.9
Biphenyl Dioxygenase	−	+	−
Biphenyl Dihydrodiol Dehydrogenase	+	+	−
Biphenyl Catechol Oxygenase	+	+	−
Hydrase	+	+	−

[a] Fragments (in kb) produced by *Eco*RI Digest of pGEM410

SUMMARY

Major accomplishments for the past year are

1. Construction of genomic libraries of *P. putida* LB400 and *A. eutrophus* H850.
2. Identification and isolation of five recombinant *E. coli* strains that express LB400 genes encoding enzymes for PCB/biphenyl metabolism.
3. Demonstration that several of the recombinant strains contain the genes for at least the first four enzymes of the PCB pathway.
4. Identification of a 6.6-kilobase *Eco*RI fragment of DNA that contains three of the first four enzymes of the PCB/biphenyl pathway. Strains containing both the 6.6- and 2.9-kb *Eco*RI fragments also express biphenyl dioxygenase activity.

FUTURE PLANS

The subcloning studies on pGEM410 will be continued in order to minimize the size of the DNA fragment encoding the biphenyl/PCB metabolic pathway. Once this is accomplished the location of the genes on the plasmid will be mapped and their DNA sequence determined. From the DNA sequences it will be possible to determine the amino acid sequences of the enzymes involved and the structure and location of regulatory regions that control the expression of the genes for PCB degradation. This work will also help to establish whether LB400 contains multiple PCB/biphenyl dioxygenases and will ultimately provide a basis for enhancing the ability of our organisms to degrade PCBs and their recalcitrant metabolites.

Chapter 5

REDUCTIVE DECHLORINATION OF PCBs IN ANAEROBIC MICROBIAL COMMUNITIES

James M. Tiedje, Stephen A. Boyd, and John F. Quensen
Department of Crop and Soil Sciences
Michigan State University
East Lansing, Michigan

INTRODUCTION

The polychlorinated biphenyl (PCB) congener distribution patterns for anaerobic sediment samples from the upper Hudson River [Brown et al., 1984] and Waukegan Harbor [Stalling, 1982; J.F. Brown, Jr., personal communication] suggest that the preferential reductive dechlorination of some of the more highly chlorinated congeners may be occurring in those environments. It is believed that a biological process must be involved because congener selectivity is observed, and no known nonbiological agents having sufficient reducing power can exist in this sediment [Brown et al., 1984]. Others have suggested that the loss of higher chlorinated congeners may be explained by selective partitioning of these congeners. Thus independent experiments that directly demonstrate biological dechlorination are important to clarify the mechansim and to make sound judgments on PCB cleanup schemes.

We have demonstrated microbially mediated anaerobic dechlorination of a variety of aromatic compounds including chlorobenzoates [Suflita et al., 1983; Shelton and Tiedje, 1984], chlorophenols [Boyd et al., 1983; Boyd and Shelton, 1984], chlorobenzenes [Tiedje et al., 1987; Fathepure et al., 1987], pentachlorophenol [Mikesell and Boyd, 1985], 2,4,5-T (2,4,5-trichlorophenoxyacetic acid) [Mikesell and Boyd, 1985; Suflita et al., 1984], and 2,4-D [2,4-dichlorophenoxyacetic acid, [Mikesell and Boyd, 1985]. Here we report on progress in conducting experiments to demonstrate biological, anaerobic dechlorination of selected PCB congeners and our plans for future work in this area.

Our basic experimental procedure consisted of adding a mixture of five PCB congeners (2,4,3′,4′-CB, 2,3,4,5,6-CB, 2,4,6,2′,4′,6′-CB, 2,3,4,6,2′,4′,5′-CB, and 2,3,4,5,2′,3′,4′,5′-CB) in 50 µl of acetone to 50 ml of anaerobic Hudson River sediment or sewage sludge in tightly stoppered serum bottles. These congeners were selected so that the relative rates of dechlorination as a function of the degree of chlorination could be compared, and so that all primary (single) dechlorination products would be resolved from all parent PCBs and, in general, from each other. The sludge used was from the sewage treatment plant in Jackson, Michigan and was used immediately ("fresh") or after acclimation to each of the (*ortho-*, *meta-*, and *para-*) monochlorophenols. Autoclaved (sterilized) sludge and sediment served as

controls, and each treatment was replicated twice. The PCB mixture was added to yield a final concentration of 1 ppm (w/w) of each congener. Samples taken at zero time and after 1, 4, 8, 16, 32, and 51 weeks of incubation were extracted with 15% methylene chloride in hexane and analyzed by capillary gas chromatography for the appearance (or increase in size) of peaks associated with the potential primary dechlorination products.

At the time of last year's report we had completed data analysis for only two congeners (2,3,4,5,2',3',4',5'-CB and 2,3,4,6,2',4',5'-CB) at incubation times up to 4 weeks. At that time we concluded that there was no evidence for PCB dechlorination by any of the sewage sludges and little if any significant dechlorination (<1% in 4 weeks) by the Hudson River sediments for the two congeners examined.

RESULTS AND DISCUSSION

Hudson River Sediments

Evidence for PCB dechlorination by the Hudson River sediments is summarized in Table 5-1. No dechlorination products from 2,3,4,5,2',3',4',5'-CB were ever observed, so it was used as the internal standard. This permitted comparison of the average percent reduction for the other parent congeners between the 8- and 51-week sampling periods. (Comparisons to earlier samples cannot be made at this time because the chromatographic column was changed between the running of the 0- to 4-week samples and the 8- to 51-week samples. There was enough difference in the resolution of the two columns to affect the integration of the peaks. All

Table 5-1

DISAPPEARANCE OF ADDED PCB CONGENERS AND APPEARANCE OF DECHLORINATED PRODUCTS *AFTER INCUBATION OF HUDSON RIVER SEDIMENTS*

PCB Congener Added	Average % Reduction[a] of PCB		Average % of Original PCB Identified As Products
	Sterile	Alive	
2,3,4,5,2',3',4',5'	std	std	0
2,3,4,6,2',4',5'	0.9	12.4	4.1
2,4,6,2',4',6'	−14.6	7.4	?
2,3,4,5,6	−0.2	43.8	≥28.0
2,4,3',4'	−13.3	31.0	?

[a] The difference in mean quantities between 8 and 51 weeks, as a percent of the original amount.

Hudson River sediment samples are being rerun by G.E. analysts to permit comparisons between all sampling periods.) For the sterile controls none of the other four PCBs added decreased over time. But for the live sediments, the 2,3,4,5,6-CB and 2,4,3',4'-CB showed notable decreases. Furthermore, dechlorinated products were detected for the 2,3,4,6,2',4',5'-CB and the 2,3,4,5,6-CB congeners.

The 12.4% decrease in the 2,3,4,5,2',4',5'-CB congener reported in Table 5-1 may not be significant, because the other added congeners showed apparent increases of similar magnitude in the sterile controls. However, a small peak corresponding to the potential *meta*-dechlorination product 2,4,5,2',4',6'-CB, and representing about 4% dechlorination, was first noted in the 32-week live samples but never in the controls.

There was no good evidence for the dechlorination of the 2,4,6,2',4',6'-CB congener. The 7.4% reduction reported in Table.5-1 is not significant, and no relative increase in peak area for either of the two potential primary dechlorination products could be detected.

The 2,3,4,5,6-CB provided convincing evidence for PCB dechlorination. This congener decreased 43.8% in concentration and showed a corresponding increase in dechlorination products. The potential *meta*- (2,3,4,6-CB) and *para*- (2,3,5,6-CB) dechlorination products coelute on the GC column used, so that it was not possible to distinguish whether one or both were formed. The resulting peak first appeared in the 8-week live samples and then increased steadily in size with time. At the end of 51 weeks it accounted for 28% of the parent penta-chlorobiphenyl. There was no evidence of the *ortho*-dechlorination product (2,3,4,5-CB). However, the tetrachlorobiphenyl formed may have undergone further dechlorination. One potential product, 2,4,6-CB, coelutes with a contaminant, but the contaminant diminished with time in both the live and sterile treatments and had nearly disappeared in the 51-week controls. It may be that the remaining peak in the 51-week live samples was caused by 2,4,6-CB rather than the contaminant. Similarly, the potential dechlorination product 2,6-CB coelutes with 2,2'-CB, but this peak is larger in the live 51-week samples than the corresponding controls. Thus more than 28% of the original 2,3,4,5,6-CB can be accounted for by products.

The 2,4,3',4'-CB is apparently being dechlorinated, because there is a steady decrease with time in the peak size relative to 2,3,4,5,2',3',4',5'-CB in the live samples, but the detection of products is questionable because of high background levels and/or poor resolution for the expected trichlorobiphenyls.

Additional indications of dechlorination appear in the Hudson sediment experiments if one examines changes in the relative peak sizes for the background levels of PCBs. For example, in the 51-week samples the peak for 2,4,2'-CB is relatively larger in the live samples than in the controls. The peak for 2,6,4'-CB is larger relative to the peaks for 2,5,3'-CB and 2,5,4'-CB in the live samples than in the controls. Similarly the peak for 2,4,2',4'-CB is larger relative to the peaks for 2,5,2',5'-CB and 2,4,2',5'-CB in the live samples.

Sewage Sludge Experiments

PCB dechlorination by the Jackson sewage sludge was much more limited. In fact, no primary dechlorination products were detected in any of the sludges acclimated to the chlorophenols. The fresh (unacclimated) sludge did show a small amount of 2,3,4,6,2′,4′-CB first appearing in the 8-week samples, and accounting for the dechlorination of about 2% of the 2,3,4,6,2′,4′,5′-CB added. This peak did not increase substantially after 8 weeks.

SUMMARY AND CONCLUSIONS

Of the five PCB congeners added to Hudson River sediments, two (2,3,4,5,6-CB and 2,3,4,6,2′,4′,5′-CB) were dechlorinated, as evidenced by the accumulation of products, and a third (2,4,3′,4′-CB) was likely dechlorinated, based on significant disappearance relative to the sterile controls. Some further changes in the relative amounts of the PCB congeners already present in the sediments occurred during the course of the experiment. These changes are also interpreted to be the result of reductive dechlorination by anaerobic microorganisms, since the changes did not occur in sterile controls. The most readily dechlorinated congener was the 2,3,4,5,6-CB; 43.8% disappeared during 43 weeks of the experiment.

Much less PCB dechlorination was observed in the Jackson sewage sludges. Only one dechlorination product was identified, accounting for dechlorination of 2% of the 2,3,4,6,2′,4′,5′-CB originally added. Since this sludge was the most active in dechlorinating other substrates, e.g., chlorophenols and hexachlorobenzenes, the long-term Hudson River PCB exposure may have resulted in selection for PCB dechlorinators.

Our data strongly suggest that all of these changes are biologically mediated, as they were not observed in the sterilized controls. Also, all changes involved the removal of *meta*- or *para*-chlorines only. This is consistent with the environmental data [Brown et al., 1984, GE Reports 1984, 1985, 1986] showing the accumulation of only *ortho*- substituted chlorobiphenyls.

FUTURE PLANS

Objectives for our future work are

1. To evaluate the PCB-dechlorinating activity of sediments from other sites.
2. To evaluate the dechlorination of other congeners, especially those of greater toxicological importance.
3. To enhance the rate of dechlorination

Experiments are currently under way to evaluate the PCB dechlorinating ability of other Hudson River sediments, sediments from Silver Lake, Massachusetts (containing PCBs) and Pine River, Michigan (containing polybrominated biphenyls and hexabromobenzene), and from Red Cedar River, Michigan (below a sewage treat-

ment plant but without significant PCB exposure). These studies are being conducted using a mixture of selected PCB congeners as before. To avoid the problem of detecting dechlorination products against the high background PCB levels present in some of these sediments, the microorganisms have been selectively eluted and transferred to cleaner sediments. Some sediment must be present to bind the PCBs. We have found that the less chlorinated congeners selectively partition to the stoppers during long incubations of highly enriched sediment-free cultures.

The PCB mixture currently being used includes 3,4,3',4'-CB instead of the 2,4,3',4'-CB previously used. This will allow the evaluation of a non-*ortho*-substituted PCB. Furthermore, 3,4,3',4'-CB is the most toxic isomer present in Aroclor 1242. This Aroclor was the major PCB discharged to the Hudson River sediments.

Experiments are also planned to add Aroclor 1242 or Aroclor 1260 to anaerobic sediments at various concentrations. This will not only allow us to determine which congeners are most readily dechlorinated but will also allow us to examine the effect of PCB concentration on the dechlorination rate. If the dechlorination reaction provides energy to the microoganisms involved, then a suitable PCB concentration may actually select for the dechlorinating agent. Our original thinking was that PCB dechlorination is most likely fortuitous. However, recent experiments with an isolate that dechlorinates chlorbenzoates, DCB-1, indicate that this organism derives energy from the dechlorination [Dolfing and Tiedje, 1987]. Calculations show that the dechlorination of PCBs could provide a comparable energy yield.

A major problem with long-term incubations is that biological activity decreases with time as nutrients are consumed. We have been conducting experiments with chlorophenol-degrading enrichments and ^{36}Cl-tetrachlorodibenzo-*p*-dioxin to determine what amendments are most suitable for supporting dechlorination during long-term incubations. Ethanol, Triton X-100, and hematin have been found to inhibit dechlorination. Acetate, sludge extract, and yeast extract stimulated dechlorination. Experiments using PCBs in sediment systems have been initiated to test the effects of these last three amendments on the rate of PCB dechlorination. Triton X-305 will be used in another treatment to test the effect of this solubilizer on the PCB dechlorination rate; Triton X-305 has been shown to be less toxic to anaerobes than Triton X-100 [GE Report, 1986].

These experiments will provide estimates of the rate of dechlorination in amended sediments and information on how the rate can be increased. This information is important for deciding what action is required to deal with contaminated sediments and may also be of use in developing a biological anaerobic treatment process.

Chapter 6

ENVIRONMENTAL TRANSFORMATIONS OF PCBs

John F. Brown, Jr.
Biological Sciences Branch

Robert E. Wagner and James C. Carnahan
Organic and Polymer Characterization Unit

General Electric Corporate Research and Development
Schenectady, New York

INTRODUCTION

Until recently, it was believed that the more heavily chlorinated PCB congeners, e.g., the major components of Aroclors 1254 and 1260, were essentially indestructible in the environment [Furukawa, 1982]. Then, in 1984, it was reported [GE Report, 1984; Brown et al., 1984] and immediately confirmed [Bopp et al., 1984] that the higher PCBs in the sediments of the upper Hudson River (Hudson Falls, New York to Troy, New York) were undergoing reductive dechlorination. The evidence for this chemical change in the PCB residues lay in their gas chromatograms. These chromatograms showed marked declines in the levels of the hexa-, penta-, tetra-, and most trichlorobiphenyl congeners present, and corresponding increases in the levels of the mono-, di-, and certain minor trichlorobiphenyls. Furthermore, the patterns of higher congener loss and lower congener gain were not identical in all sediment samples. Several repeatedly observable patterns, which were given letter designations in approximate order of their discovery, were noted [Brown et al. 1984]. Evidently, not only was PCB dechlorination occurring in the upper Hudson, but several distinguishable agents (now known to be different populations of anaerobic bacteria) were responsible for the process.

Since 1984, we have been working steadily to broaden the scope of this discovery. It was soon found that the upper Hudson sediments contained not only the three dechlorination systems originally reported (Systems B, B′, and C) but also a fourth (System E) that was particularly effective in attacking the small amounts of hexa-, hepta-, and octachlorobiphenyls present. Investigations of the sediments of Silver Lake (Pittsfield, Massachusetts), which had received considerable quantities of Aroclor 1260, revealed the presence of two more dechlorination systems: System F, which converted the higher congeners to tri- and tetrachlorobiphenyls, and System G, which took at least part of them all the way to mono- and dichlorobiphenyls. Next, the chromatograms that had been reported [Stalling, 1982] for the Aroclor 1248 residues in the harbor sediments of Waukegan, Illinois were found to exhibit marked transformation by some different dechlorination agent, which we designated System W. Evidences for dechlorination by systems that may or may

not be identical to any of the above were also seen in the chromatographic data for sediments from the Sheboygan River (Sheboygan, Wisconsin), the Hoosic River (North Adams, Massachusetts), and Massena, New York, which were sent to us by other investigators. Most of these findings are summarized in previous reports of this series [GE Reports, 1985 and 1986]. Formal scientific reports will be published shortly [Brown et al., 1987a; 1987b].

During the past year we have continued to explore the scope of this environmental dechlorination phenomenon, with particular emphasis on its occurrence at marine sites, which had been previously neglected.

RESULTS AND DISCUSSION

Achusnet Estuary PCBs

The Acushnet River estuary (New Bedford, Massachusetts) has received extensive releases of Aroclors 1242 and 1254 from two capacitor manufacturers: Cornell-Dublier, located on the lower estuary (outer harbor), and particularly from Aerovox, located on the upper estuary [Weaver, 1984]. The PCB levels in the local sediments, water, molluscs, crustaceans, and finfish have been extensively reported [Metcalf and Eddy, Inc., 1983].

Our review of the available chromatograms indicated little evidence for environmental PCB transformations (other than some nonselective loss of lower congeners) in the sediments of the lower estuary, but considerable evidence of higher congener depletion in those of the upper and middle estuaries. Accordingly, upper estuary mudflat sediments were collected at low tide from six sites each along both the east and west sides of the estuary, and from two depths (2 to 3 in. and 6 to 7 in.) at each site. The resulting 24 sediment samples were analysed as previously described [Brown et al., 1984; 1987b] to provide high resolution DB-1 capillary gas chromatograms and quantitative PCB levels for all 118 resolvable PCB congener peaks. In addition, gas chromatographic-mass spectrometric (GC-MS) ion chromatograms were obtained on four samples representing different stages of the observed transformations.

Analysis of these data indicated (a) that there was no evidence for the presence of Aroclors other than 1242 and 1254 (e.g., Aroclors 1221, 1016, 1248, or 1260) in the composition received by the sediments, and (b) that the original Aroclors had subsequently undergone three types of alteration. These alterations consisted of a small, nonselective loss of lower congeners, undoubtedly as a result of extraction into the water column, and dechlorination by two very similar dechlorination processes, which were designated H and H′. Process H′ appeared at just two adjacent sites on the west side of the estuary; Process H was seen at all the others. Figure 6-1 shows DB-1 capillary gas chromatograms for specimens exhibiting well advanced Process H and H′ transformations, along with reference chromatograms for Aroclor 1016, Aroclor 1242, Aroclor 1242 depleted of lower congeners by evaporation, and Aroclor 1254. Figure 6-2 shows the corresponding GC-MS patterns for

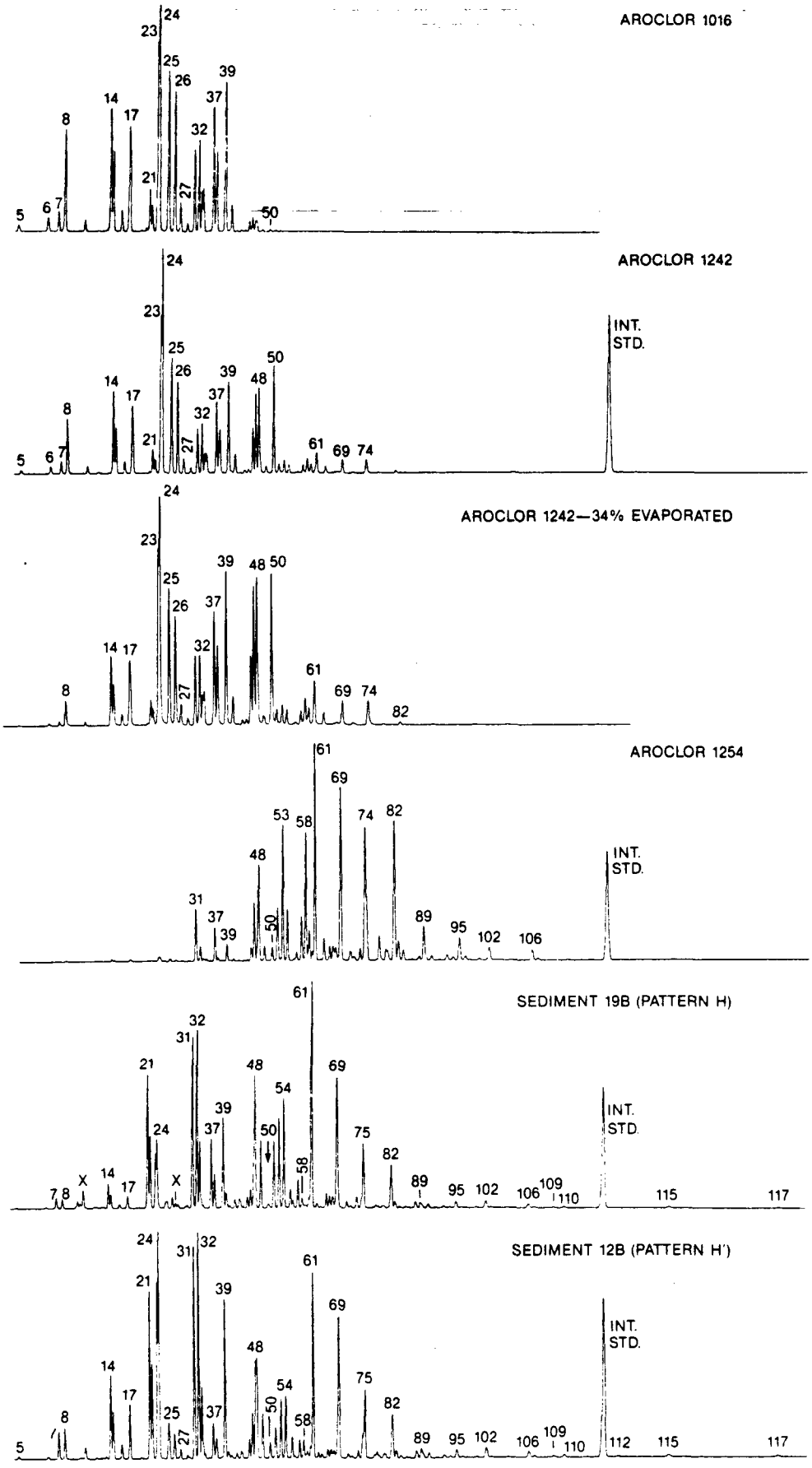


Figure 6-1. **DB-1 capillary gas chromatograms of Aroclor reference standards and Acushnet sediment samples exhibiting alteration patterns H and H'.**

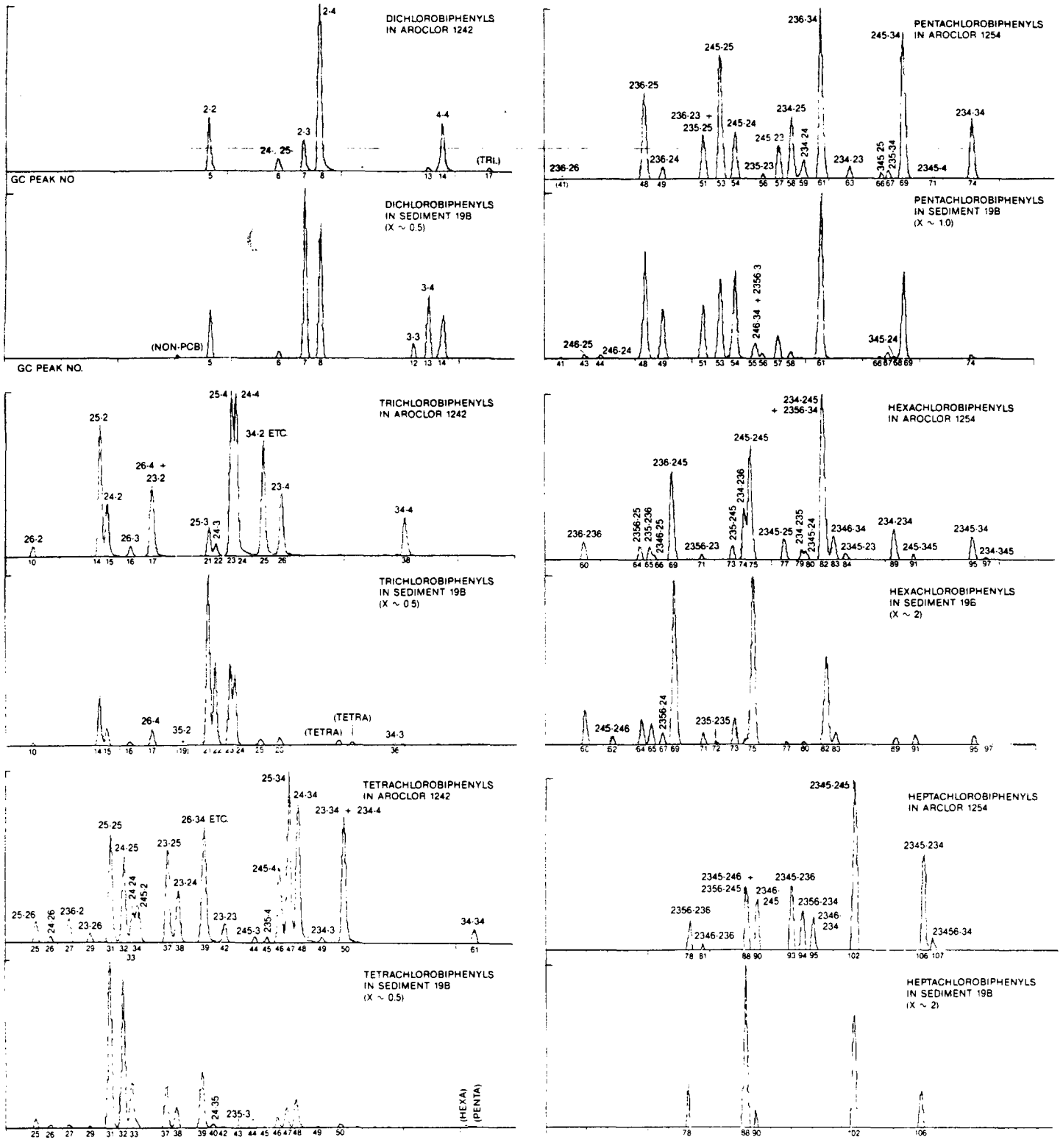


Figure 6-2. **DB-1 capillary gas chromatographic-mass spectrometric ion chromatograms, showing summed parent ion isotope peaks for di- through heptachlorobiphenyls in Aroclor standards and Acushnet sediment sample 19B.**

the di-, tri-, tetra-, penta-, hexa-, and heptachlorobiphenyls present.

Analysis of the data showed that in both Process H and H′ the major transformations were the conversions of 3,4-dichlorophenyl groups to 3-chlorophenyls; of 2,3,4-trichlorophenyls to 2,4-dichlorophenyls; and of 2,4,5-trichlorophenyls somewhat more slowly to 2,5-dichlorophenyls. Thus, the dichlorobiphenyl tracings of Figure 6-2 show increases in 2,3′-, 3,3′-, and 3,4′-CB and the trichlorobiphenyl tracings marked increases in 2,5,3′-, 2,4,3′- and 3,4,3′-CB with corresponding decreases in 3,4,4′-, 2,5,3′,4′-, 2,4,3′,4′-, 2,3,3′,4′-, and 3,4,3′,4′-CB. Likewise, there were marked decreases in 2,3,4,2′,5′-, 2,3,4,2′,4′-, 2,3,4,2′,3′-, 2,3,4,3′,4′-, 2,3,4,2′,3′,6′-, 2,3,4,2′,4′,5′- and 2,3,4,2′,3′,4′-CB with corresponding increases in 2,4,2′,5′-, 2,4,2′,4′-, 2,3,6,2′,4′-, and 2,4,5,2′,4′-CB. Less abundant chlorophenyl (CP) groups undergoing significant decline included 2,3-, 2,3,5-, 3,4,5-, 2,3,4,5-, 2,3,4,6-, and 2,3,4,5,6-CP; and the new chlorophenyl groups formed included small amounts of 3,5-, 2,4,6-, and presumably also 2- and 2,3,5,6-CP, in addition to 3-, 2,4-, and 2,5-CP. System H′ differed from H in its greater rate of attack on 2,3-dichlorophenyl groups, and also a slight activity toward 2,3,6-CP. Otherwise, neither H nor H′ appeared to attack monochlorophenyl groups; dichlorophenyls other than 2,3- and 3,4-CP; 2,3,6- or 2,4,6-trichlorophenyls, or 2,3- or 3,4-dichlorophenyls in the presence of 2′,6′- substitution. The H/H′ dechlorination system thus appears to be somewhat more selective in its congener removal pattern than those discovered earlier. Nevertheless, the congeners of toxicological concern, such as 3,4,3′,4′-, 2,3,4,3′,4′, 2,3,4,5,4′-, and 2,3,4,5,3′,4′-CB, are all strongly attacked, so that detoxication is effected. Moreover, the very selectivity of the system imparts highly characteristic features to the gas chromatogram (enhanced peaks for 2,3′-, 2,5,3′-, 2,4,3′-, 2,5,2′,5′-, 2,4,2′,5′-, 2,4,2′,4′-, 2,3,6,2′,4′-, and 2,4,5,2′,4′-CB; sharply diminished peaks for 2,4,5,4′- and 2,5,3′,4′-CB, and for all congeners containing 2,3,4- or 2,3,4,5-chlorophenyl groups) which facilitates recognition of the H/H′ alteration process in environmental PCB mixtures.

Within the New Bedford area, Pattern H/H′ could be seen in the sediments of the upper and middle estuaries, but not those of the lower estuary (outer harbor). It also appeared strongly in most, but not all, upper estuary water samples, in some water samples of the lower estuary, and in lower estuary molluscs. (In lower estuary crabs and lobsters, any dechlorination pattern present was overriden by the endogenous metabolism, resulting in a congener depletion pattern that closely resembled those seen in warm-blooded animals.) Elsewhere, patterns resembling H or H′ were seen in one of two sediment samples from Escambia Bay (Pensacola, Florida) and in several fish from the greater New York metropolitan area. Thus, the H family of patterns may prove to be generally characteristic of PCBs in marine dechlorination sites.

Housatonic River PCBs

The Housatonic River originates in the Berkshire Mountains of western Massachusetts near Pittsfield, Massachusetts, and flows south through western Mas-

sachusetts and Connecticut, a little east of the New York state line. Its flow is interrupted by several dams and impoundments, notably including Woods Pond, which is located just north of the Massachusetts-Connecticut state line. The Housatonic River is known to have received releases of Aroclors 1260 and 1254 from transformer manufacturing operations in Pittsfield. Some of these releases entered the river directly; others may have formerly entered the river via the overflow from Silver Lake, although the PCBs of the Silver Lake sediments are no longer passing on to the river [Stewart Laboratories, Inc., 1982].

The sediments of Silver Lake consist of a black, oily, methanogenic muck that may contain anywhere between 30 and 7000 ppm of PCBs. As previously noted, about half the samples collected show strong alteration by either Process F or G, both of which (unlike dechlorination processes B, B', C, E, W, H, or H') are capable of removing chlorines from *ortho*, as well as *meta* and *para*, positions on the biphenyl rings. Additional samples of these sediments were taken through the ice in February 1987, in part to provide System G organisms for laboratory studies by the Michigan State group, in part to permit additional chemical characterization of dechlorination system G, and in part to permit isolation of sufficiently large quantities of the dechlorinated Aroclor 1260 for toxicological studies.

The sediments of Woods Pond contain roughly 10 to 200 ppm of PCBs. Studies now in progress indicate a relatively uniform composition for these PCBs (ca. 65% Aroclor 1260, 35% Aroclor 1254, traces only of Aroclor 1242), with evidences of dechlorination by some rather selective, H-like dechlorination system rather than Silver Lake System F or G. Thus, some of the Aroclor 1254 previously reported by the analysts may represent dechlorinated Aroclor 1260.

We are also investigating the PCBs in the sediments from several Housatonic River impoundments in west-central Connecticut. These generally contain only 1 to 6 ppm of PCBs, again mainly Aroclor 1260, with some 1254 and a little Aroclor 1242, but with no obvious evidences of ongoing dechlorination.

PCBs from Other Sites

We have received from Professor J.M. O'Connor, of the NYU School of Environmental Medicine, a collection of DB-5 capillary gas chromatograms of dredging spoils from several sites in the New York-New Jersey metropolitan area, where the PCB levels in the sediments generally run in the 1 to 7 ppm range. All showed the common environmental PCB composition (roughly 65-75% Aroclor 1242, 20-25% Aroclor 1254, 5-10% Aroclor 1260), which also approximates that found in the local sewage discharges. None of these lightly contaminated metropolitan area sediments showed any obvious evidences of dechlorination. Previously, it had been reported that the upper Hudson dechlorination processes were not occurring below Albany, in the tidal Hudson [Bopp, 1984].

Our attention has recently been directed to an obscure Japanese report indicating preferential losses of the Kanechlor 300 peaks now known to correspond to

congeners 3,4,2'-, 2,5,3',4'-, 2,4,3',4'-, and 2,3,3',4'-CB in flooded paddy soil [Wakimoto et al., 1971]. The observed selectivity pattern strongly indicates anaerobic dechlorination rather than aerobic microbial metabolism. What may be most significant, however, is that the reported half-time for 2,5,3',4'-CB removal was only about 10 weeks, rather than the 3 years that we have estimated for that congener in upper Hudson sediments (Systems B, B', or C) or the 7 years estimated for the upper Acushnet (Systems H, H'). Thus, it may be that with suitable nutrition the PCB dechlorination process can be made to occur quite rapidly.

A more obvious requirement for dechlorination — or at least the initiation of a dechlorination process — is a relatively high level of PCB in the sediments. At all three sites where data is available — the Hudson River, the Acushnet Estuary, and the Housatonic River — we saw well-advanced dechlorination, albeit by different systems, in the heavily PCB-laden upstream sediments near the spill sites, and then little or no dechlorination further away, where the PCB levels were low. This behavior is consistent with our proposal that the PCB-dechlorinating agents are anaerobic bacteria that have been able to successfully compete with the other resident anaerobes by virtue of their ability to use PCB as a terminal electron acceptor. Obviously, this competitive advantage would be lost if the PCB levels were too low to permit its capture and use by these PCB-dechlorinating species.

SUMMARY

PCBs have now been found to undergo reductive dechlorination in marine, as well as in freshwater, sediments. Very similar patterns of higher congener depletion and lower congener increase were seen in sediments of the upper Acushnet River estuary (New Bedford, Massachusetts), those of Escambia Bay (Pensacola, Florida) and in certain metropolitan New York fish.

In sediments of the Housatonic River system, the PCBs (mainly Aroclor 1260) were found to be undergoing two kinds of dechlorination in Silver Lake (Pittsfield, Massachusetts), another in Woods Pond (southwestern Massachusetts), and probably none at all in the very lightly contaminated Connecticut portions of the river.

FUTURE PLANS

During the coming year we expect to devote much of our time to our ongoing studies of environmental dechlorination processes, and to the environmental factors that allow them to proceed.

In addition, we will investigate the aerobic biodegradation of PCBs in natural waters, the environmental factors controlling its rate, and possible routes to its acceleration.

Chapter 7

BACTERIAL DEGRADATION OF PCBs IN SOIL

Ronald Unterman, Ronald E. Brooks, and Michael J. Brennan
Biological Sciences Branch

David P. Mobley, John B. McDermott, and Charles C. Schwartz
Chemical Engineering Branch

Robert E. Wagner
Organic and Polymer Characterization Unit

General Electric Corporate Research and Development
Schenectady, New York

INTRODUCTION

Our initial studies with PCB-contaminated sand and soils have clearly demonstrated that PCBs on these substrates are biodegradable under laboratory conditions [GE Reports 1985, 1986; Unterman et al., 1986; Unterman et al., 1987]. Last year we began studies with actual PCB-contaminated soil from New York State and were able to demonstrate substantial biodegradation using our resting cell laboratory protocol (51% degradation in 3 days, [GE Report, 1986]). This environmental sample is from the site of a former racing drag strip where PCB oils were used for dust control. The soil contains 525 ppm of a transformed Aroclor 1242. It is depleted in the di- and trichlorobiphenyls and therefore appears similar in composition to Aroclor 1248.

For the past year we have focused our efforts on scaling up the drag strip soil study in preparation for conducting a site test at the drag strip (scheduled for summer 1987). Our plan is to inoculate a small test area (3×3 m) with *P. putida* strain LB400 and monitor for PCB biodegradation as compared to an adjacent control plot. In preparation for this test we initiated three programs: (1) preparation of the site (water, power, fencing, test plot tents, test wells, equipment, etc.) (2) scale-up culturing of LB400 to delivery up to 1000 L cell culture per week, and (3) determination of conditions and final protocol for conducting an in situ biodegradation (in contrast to our previously used resting cell laboratory protocols).

RESULTS AND DISCUSSION

The consulting engineering firm of Woodward-Clyde (Wayne, NJ), completed the site preparation in the fall of 1986. (See Figure 7-1. Note: the tent canopy will be installed just prior to the test.)

The mass culturing of LB400 has been successfully carried out on a 200 L scale. LB400 is grown using biphenyl (BP) as sole carbon source and reaches a cell density

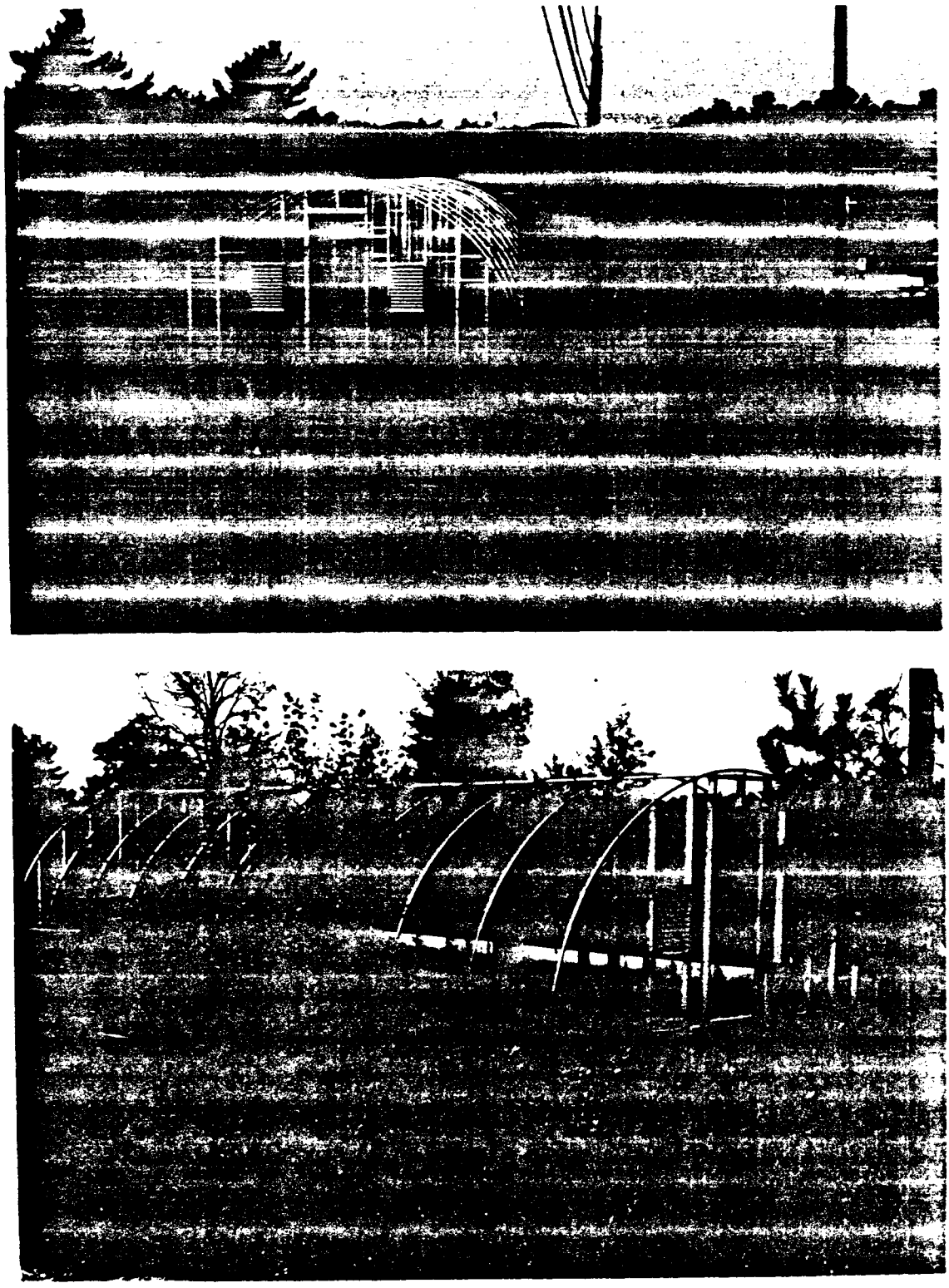


Figure 7-1. Drag strip test site.

of greater than 10^9 cells/ml in 24 h using a 1% inoculum. This rate of cell production is more than sufficient for the required bacterial dosings.

The third preparative program required our greatest efforts. A series of experiments were begun in an effort to model the conditions, scale, and procedures applicable to the full test. Thus, 1 to 5 kg samples of drag strip soil were incubated with LB400 using experimental conditions more suitable to an in situ application of bacteria. These in situ conditions (in contrast to our laboratory resting cell experiments) included 3-4% of the previously used cells, unwashed cells, less water, 8 °C cooler, no shaking, and less aeration. The experiments were designed to test parameters such as (1) rate of PCB degradation as a function of dosing frequency and bacterial concentration in the soil, (2) effect of soil mixing following bacterial application, (3) degradation rate as a function of temperature, and (4) response of various control applications (e.g., water, buffer, Hg^{+2} poisoned LB400, and a mutant strain of LB400 that cannot grow on biphenyl.) PCB biodegradation was just detectable after 30 days, a much slower rate than observed with the resting cell protocol. However, the laboratory study with drag strip soil eventually did show significant PCB biodegradation albeit in weeks rather than days. In an undisturbed soil sample (1 kg), inoculated three times weekly with LB400, 50% of the PCB in the topmost soil was biodegraded in 15 weeks (Figure 7-2). However, much less degradation (10%) was seen at depths below 1 cm. Bacterial cell counts indicated at least ten-fold fewer bacteria at these depths. When a duplicate of the undisturbed soil experiment was mixed at 3 months with continued inoculation, the redistributed soil again exhibited the greatest biodegradation rate in the surface soil. Thus mixing is an important variable and must be considered for the final protocol.

In parallel experiments, 5 kg of drag strip soil were inoculated three times weekly with LB400, but then mixed after each application. After 23 weeks, the PCBs in this soil were 35% biodegraded at all depths. Although this sample showed a lower percentage of biodegradation than the unmixed soil experiment discussed above, it actually represents a greater amount of PCB destruction because the PCBs were degraded throughout the whole sample, not just at the surface.

In an unexpected result, a control sample inoculated three times per week with heat-killed LB400 showed a small amount of PCB biodegradation. Coincident with this PCB depletion we observed the appearance of PCB-degrading bacteria in this control soil. Purification and analysis of these bacteria has shown that they differ substantially from LB400. Their PCB congener selectivity pattern is indicative of the more common 2,3-dioxygenase-type, and matches that of a bacterial isolate (strain DS100) enriched from drag strip soil independently using a standard biphenyl growth selection. It therefore appears that inoculating with cell debris (i.e., nutrients) and residual biphenyl from the culture medium may enrich the population of indigenous PCB-degrading organisms in drag strip soil. These bacteria are vastly inferior to LB400, both as determined by resting-cell assays and drag strip PCB biodegradation.

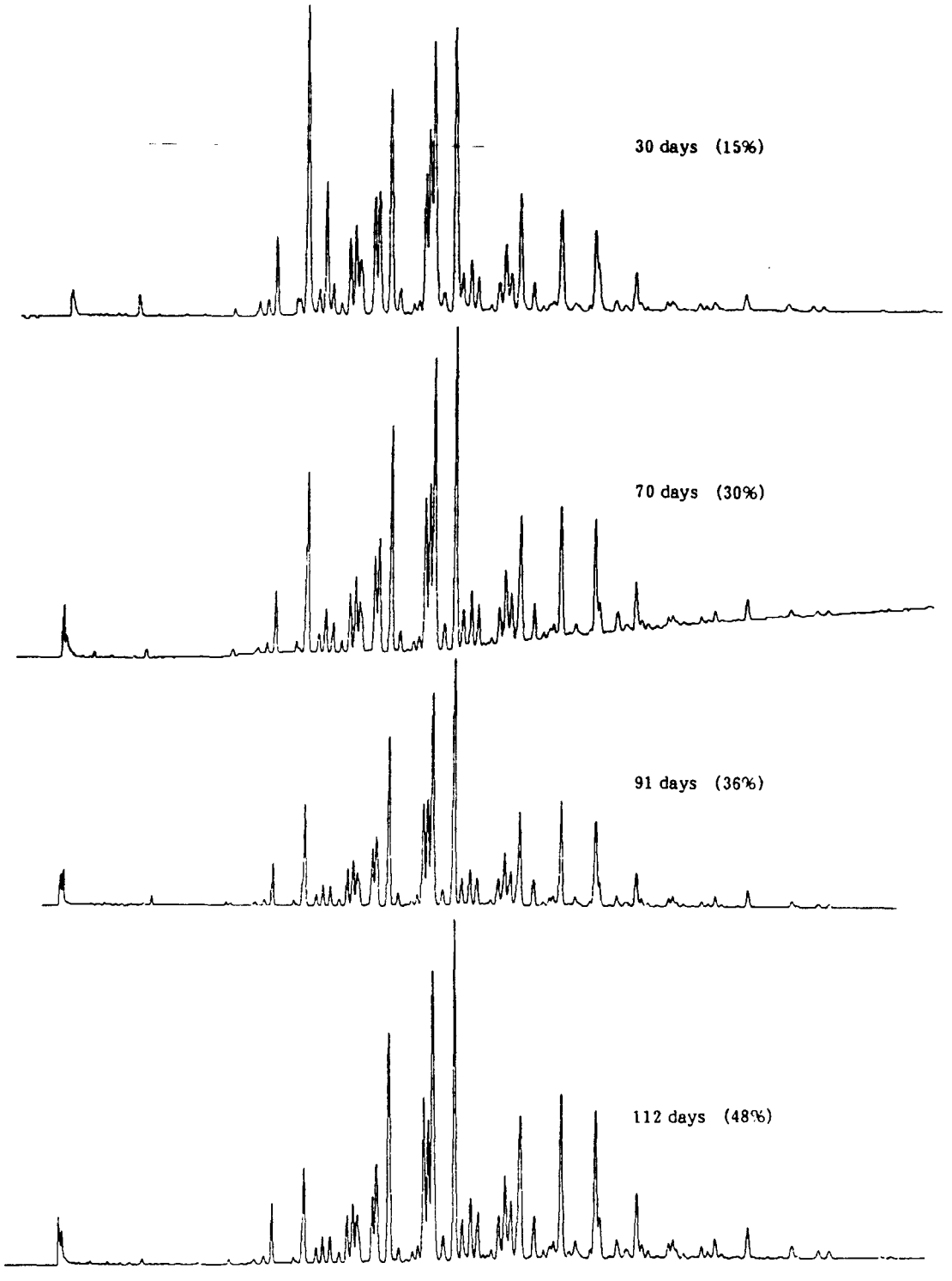


Figure 7-2. **Time course of PCB biodegradation in drag strip soil by LB400 using in situ conditions. A 1-kg soil sample was dosed three times per week with 130 ml of bacteria (2×10^9 cells/ml) and left undisturbed at ambient temperature. The panels show gas chromatograms of the PCBs extracted from surface soil (to a depth of 1 cm) collected at various times during the experiment. The numbers in parentheses indicate the total weight percent of PCB degraded based on individual peak analysis. (This is sample JU in Figure 7-3.)**

Interestingly, although the heat-killed cells (Figure 7-3, sample D) and LB400 inoculated weekly (Figure 7-3, sample F) showed the same extent of PCB degradation, they show different patterns of alteration. Sample F exhibits depletion of the same congeners (albeit to a lesser extent) as the other LB400 inoculations (Figure 7-3 samples JU and H) demonstrate; however, Sample D showed only depletion of two or three peaks, none of which are characteristic of LB400 (e.g., 2,5,2',5'-chlorobiphenyl). This is further evidence that the application of dead-cell debris provides sufficient nutrient to enrich inferior indigenous bacteria such as DS100.

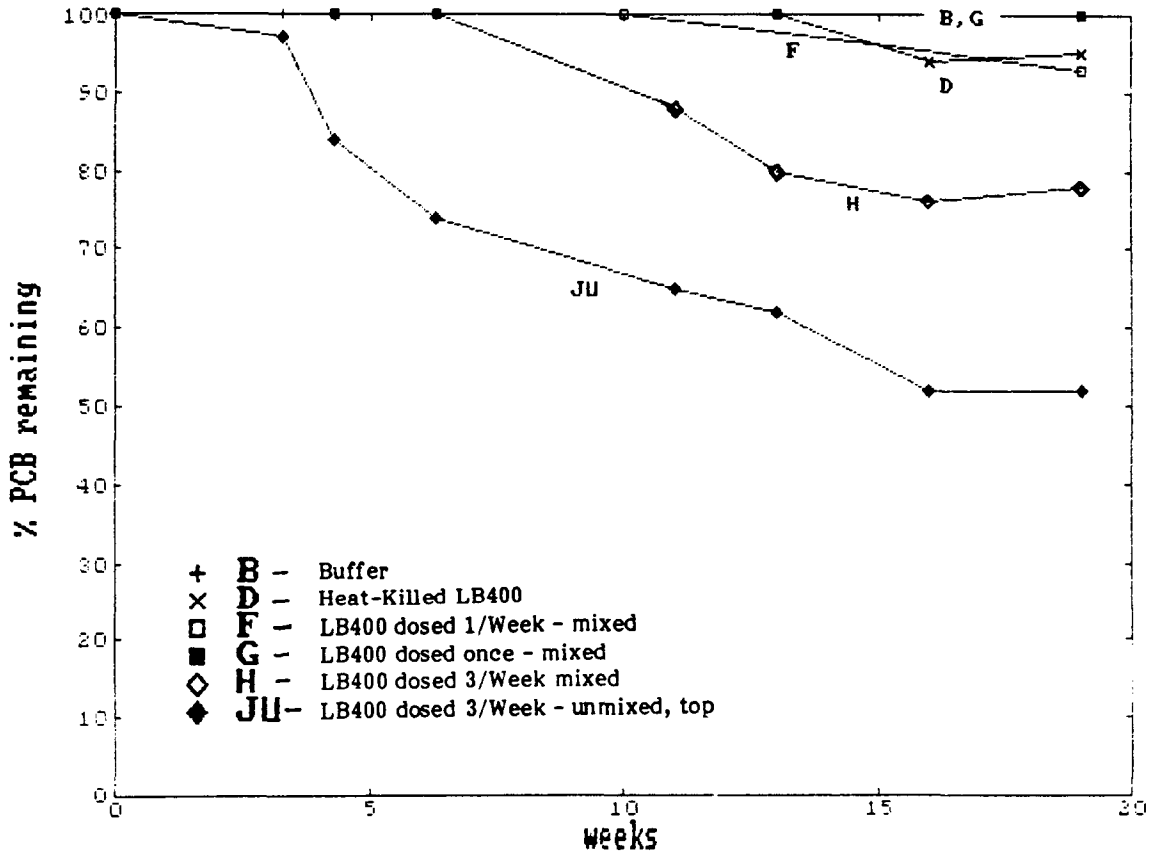


Figure 7-3. **Biodegradation of PCBs in drag strip soil using various in situ conditions and dosing schedules. Samples B, D, F, G, and H were dosed as indicated then mixed following each dosing. The soil samples were 5 kg, and the cell dose was 750 ml (2×10^9 cells/ml). See Figure 7-2 for description of Sample JU.**

SUMMARY AND CONCLUSIONS

Site preparation and mass culturing techniques for a field test have now been completed. Our model studies have made it clear that the rate of PCB biodegradation using in situ conditions is significantly slower than the resting-cell conditions conducted at higher cell concentration and higher incubation temperatures. We must, therefore, anticipate that it will take weeks instead of hours or days to see the same extent of PCB degradation in the field as that seen under optimized laboratory conditions. In addition, the frequency of dosing is very significant, probably because of the lack of extended LB400 viability in soils. Therefore, we expect to conduct a thrice weekly dosing during the site test.

FUTURE PLANS

The site test will be our major project for the coming months. In addition, we are expanding our model laboratory studies in an effort to further define the best conditions for conducting in situ PCB biodegradation. We are attempting to define the limits of PCB biodegradation using LB400 and perhaps to extend these by use of co-cultures with *Corynebacterium* sp. MB1. Eventually, we would like to conduct laboratory soil decontamination studies using superior genetically engineered PCB degrading strains as described in Chapter 4.

Chapter 8

PROCESS MODELING: SURFACTANT EXTRACTION OF PCBs FROM SOIL

David P. Mobley, John B. McDermott, and Charles C. Schwartz
Chemical Engineering Branch

General Electric Corporate Research and Development
Schenectady, New York

INTRODUCTION

Aqueous surfactant solutions have been shown to remove PCBs from contaminated soils [GE Reports 1982, 1983, 1984, 1985, 1986]. As outlined in the 1985 GE Report, our engineering program to study surfactant extraction of PCBs was divided into two phases. Phase I consisted of gathering basic materials, properties, and process data required for engineering design; Phase II involves process design, definition of process steps, equipment specification, and a bench-scale demonstration of the process.

During the first year of engineering studies (June 1985 to June 1986), we concentrated on Phase I. From these initial studies we were optimistic that an extraction process for soil decontamination was technically feasible. For example, 35 to 100% removal of Aroclor 1260 from model soils was achieved using 1 to 3 wt% aqueous surfactant solutions. The two candidate surfactants were Triton X-100 (nonionic) and Surco 233 (anionic). The two model soils studied were a PCB-free fill soil from the Oakland site and an unrefined kaolin clay, selected to model the near-surface Oakland fill layer and the subfill clay matrix, respectively. As noted in the 1986 GE Report, we subsequently found a montmorillonite clay to be a better model of the clay matrix at the Oakland site than the kaolin clay.

During the past year we concentrated on concluding Phase I, and began work on Phase II. Phase I work involved continued model soil analysis (with the montmorillonite clay), development of PCB extraction isotherms, and PCB extraction rate measurements. Some aspects of Phase II addressed were stage calculations, unit operation alternatives, possible overall processes, and equipment alternatives.

RESULTS AND DISCUSSION

Montmorillonite Clay Studies

Just prior to the publication of the previous report [GE Report, 1986], we determined the subsurface soil of the Oakland site to be predominantly a montmorillonite clay. Since that time we have obtained a refined montmorillonite, Emathlite 600, from Mid-Florida Mining Company for use as a model soil system. It should be pointed out that this refined montmorillonite clay represents an extreme or exaggerated case, since the Oakland site is not composed of pure clay. Our studies have

shown the behavior of the site soil to be qualitatively similar to the Emathlite and quantitatively intermediate between the Emathlite and the other model soils (a kaolin clay and Oakland surface soil).

The most interesting feature of the Emathlite clay is its large capacity for the adsorption of the nonionic surfactant Triton X-100. The Emathlite will adsorb up to 15% of its own weight in Triton X-100. The amount of Surco 233, the anionic surfactant, adsorbed under similar conditions is less than 1%.

PCB Extraction Studies

The extraction behavior of the two candidate surfactants was studied using the model soils spiked to various Aroclor 1260 concentrations plus soil from the Oakland RD-1 core sample that had an Aroclor 1260 concentration of 1350 ppm. This information is compiled and presented in Figures 8-1 and 8-2 as two series of isotherms, one for each surfactant. An isotherm depicting complete removal of the PCB would lie horizontally on the x-axis. The opposite case, an isotherm depicting no removal, would lie vertically along the y-axis. Next to each isotherm are two numbers and a letter. The first number is the equilibrium concentration (weight percent) of surfactant in solution. The next number, in parentheses, is the surfactant concentration in the solution before adsorption onto the soil. The letter indicates whether the soil was Oakland surface soil (O) or Emathlite (E). The RD-1 extractions are depicted as single points in the figures with the starting surfactant concentration noted.

The effect of the adsorption of the Triton X-100 onto the Emathlite 600 is obvious from the nearly vertical isotherms and low equilibrium surfactant concentrations. This phenomenon renders the Trition X-100 very ineffective when used with certain clays. Extractions of RD-1 soil with Triton X-100 resulted in poor removal, though not as poor as the cases with Emathlite.

The Surco 233, on the other hand, performed rather well with all soils studied. This can be attributed to the low adsorption of Surco 233 onto any of the soils, thus leaving most of the surfactant in solution to solubilize the PCBs. This ability to perform well even with difficult clay soils makes Surco 233 or a similar anionic surfactant the best choice for the Oakland site.

Stage Calculations

The isotherms discussed above can be used to easily calculate the number of equilibrium stages necessary to effect the desired cleanup. These calculations account for the equilibrium behavior, the solid/liquid mass ratios and the efficiency of the solid/liquid separation. As an example, 1000 ppm PCB Oakland surface soil can be cleaned to 3.5 ppm with three equilibrium stages, using a 2% Surco 233 solution with a solid/liquid mass ratio of 1:4 and 70% solids underflow from the solid/liquid separator at each stage.

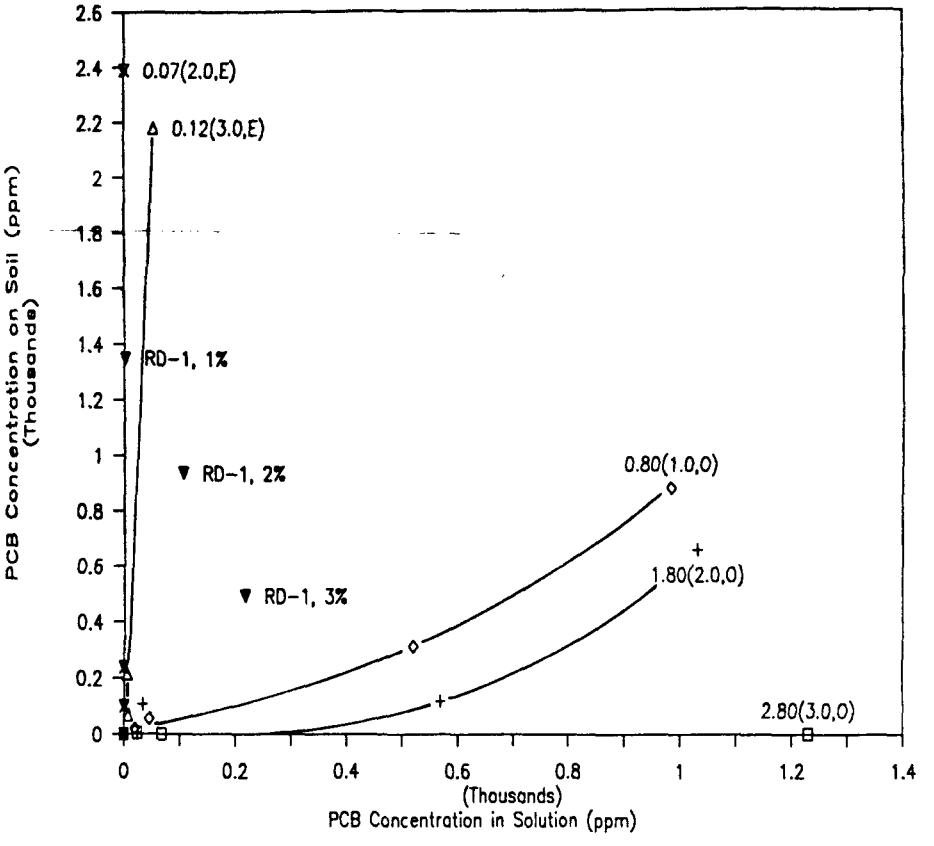


Figure 8-1. Extraction isotherms of Triton X-100.

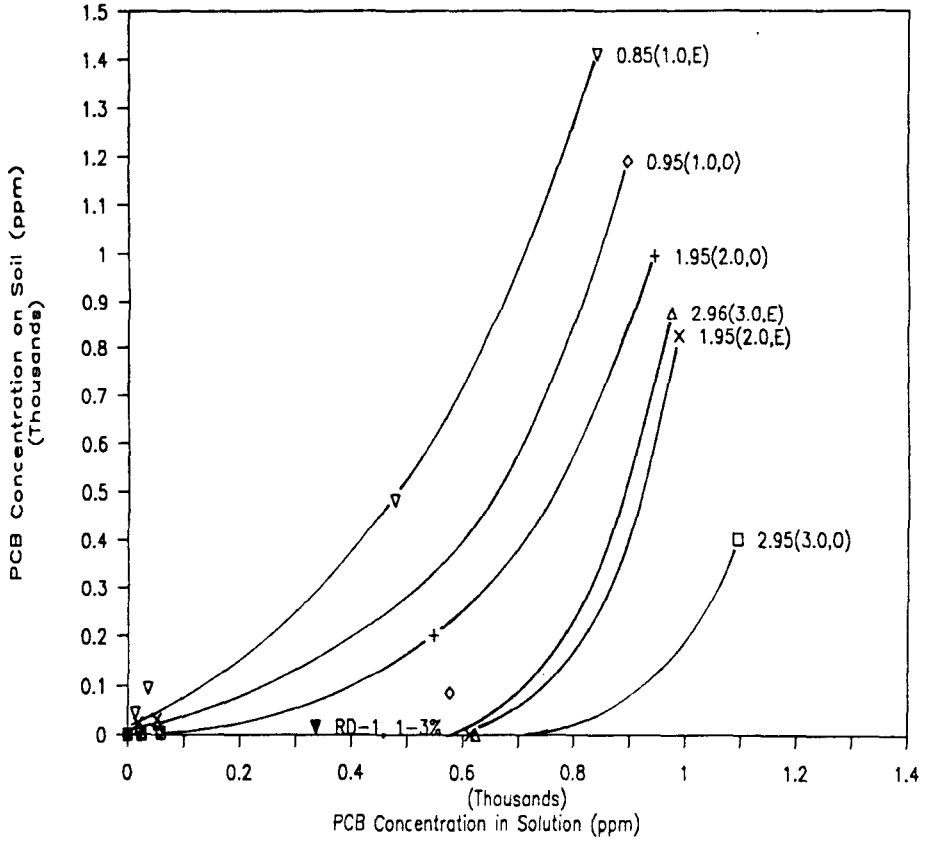


Figure 8-2. Extraction isotherms of Surco 233.

Extraction Rate

Another important consideration, besides the equilibrium behavior of the surfactant/soil/PCB system, is the rate of approach to this equilibrium state. Figure 8-3 shows the temporal variation of the solution PCB concentration normalized to its final value for an extraction of Oakland surface soil spiked to 1000 ppm Aroclor 1260. After approximately 20 min the system had attained 80% of its equilibrium value. This modest rate of approach to equilibrium results in either an increase in the number of actual stages in a short contact time process (the stages calculated above are equilibrium stages) or a longer residence time in each stage. This is typical of the tradeoffs that are encountered in the development of any process.

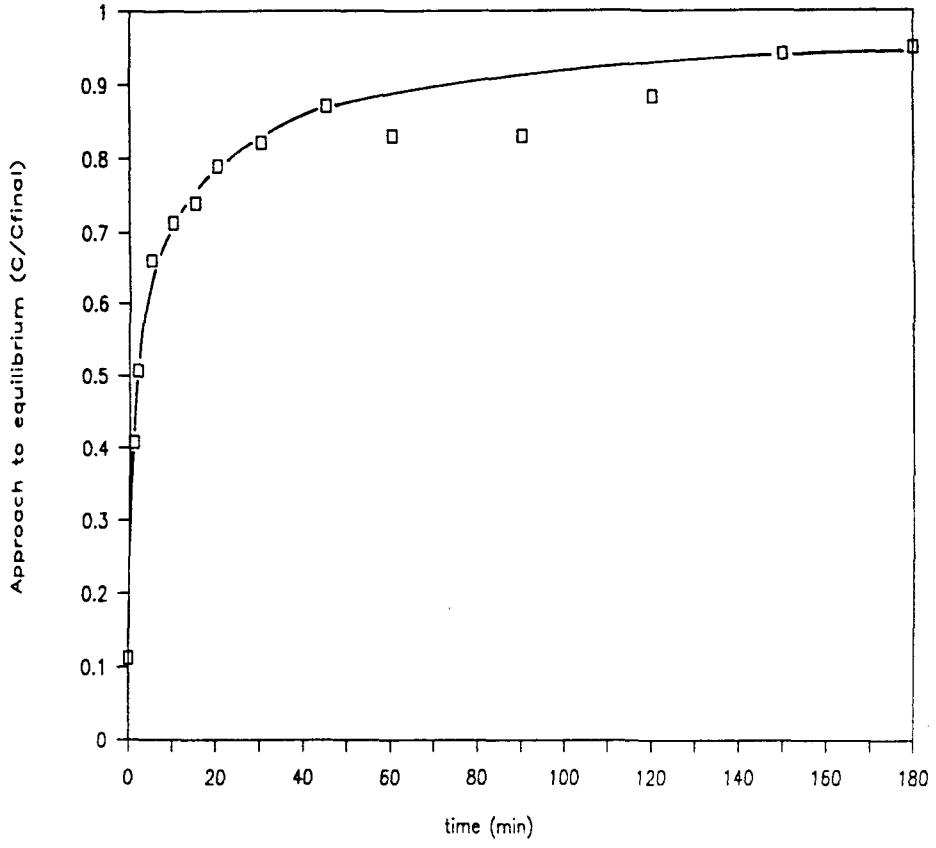


Figure 8-3. Extraction rate data for Surco 233 with Oakland surface soil.

Solid/Liquid Separation

Each extraction stage comprises a contactor and a separator. The mixing of soil and surfactant solution can be accomplished by numerous commercially available systems and presents no significant difficulty to process development. The separation of the soil from the solution after mixing is not as straightforward, because of the nature of the soil involved. Three common types of solid/liquid separators are being considered: filters, thickeners (settling tanks), and centrifuges. Each of these process options has advantages and disadvantages.

Clays, particularly montmorillonite clays, are very difficult to filter. The clay particles form virtually impermeable filter cakes even when the thickness of that cake is small. As a result, the filtration rate drops dramatically. Use of flocculants and coagulants might improve the filterability and are being investigated.

The Oakland soil exhibits a low settling rate, resulting in a low mass flux in a thickener. For example, at the relatively low processing rate of 50 tons of soil per day (30 yd^3/day), the thickener diameter would have to be 120 ft. Each extraction stage would need its own thickener. If four or more stages are called for, the area of the site occupied by thickeners could be excessive. Here again, flocculants and coagulants might increase the settling rate and reduce the area required.

The Oakland soil can be separated by centrifugation. The disadvantages of this are the cost of the equipment and the high maintenance requirements.

Process Stream Treatment

Once the soil is successfully decontaminated and returned to the excavation site, there is still the issue of the PCB-contaminated surfactant solution. The ideal treatment process would be one that removes the PCBs from the solution and allows the surfactant to be recycled back to the extractor.

To this end, we investigated numerous adsorbents in the hope of finding one that had a large capacity for PCBs while letting a majority of the surfactant pass through. We found no adsorbents that met both criteria.

Another possibility is the use of liquid/liquid extraction to remove the PCBs from the surfactant stream. The concern with this strategy is the back-extraction of organic solvent into the aqueous phase that is being recycled back to the extractor. This could result in an unacceptable amount of solvent deposited on the soil.

Solvent sublation, a method of removing organics from aqueous systems, was investigated by Dr. Sandra Woods and Li Tang. The results of their work are presented in the following chapter.

Biotreatment, while a long range goal, is not currently feasible because of the lack of an Aroclor 1260 biodegradation system.

If the ideal of recycling the surfactant cannot be easily realized, then the next best option would be the isolation of the surfactant with the PCB allowing recycling of the water. Anionic surfactants can be precipitated with divalent ions such as calcium. The formulation of Surco 233 prevents complete precipitation of the active ingredients with calcium ions. For this reason, we used the main active ingredient of Surco 233, sodium dodecylbenzenesulphonate (SDBS), for testing this strategy. When the extractive capability of SDBS was compared to that of Surco 233, there was little difference.

SDBS solutions having 600 to 1200 ppm Aroclor 1260 were precipitated with a two times stoichiometric excess of $CaCl_2$. The resultant supernatant had less than 100 ppb PCB (the lower detection limit of the analysis used). Larger quantities of fluid will be needed to accurately determine this small residual PCB.

A flowsheet of the process stream treatment is shown in Figure 8-4. The SDBS solution with PCB is mixed with $CaCl_2$ and the PCB-laden precipitate is removed and sent to disposal. The supernatant, which is an aqueous solution of salts, must undergo further treatment before recycling to remove the excess calcium ions. Failure to do this would result in the precipitation of fresh SDBS before it had an opportunity to extract any PCBs. Two options for calcium removal are ion exchange and reverse osmosis. Both of these options produce small volumes of wastewater contaminated with low levels of PCB that would have to be disposed of properly. Reduction of the volume of wastewater is possible by evaporation.

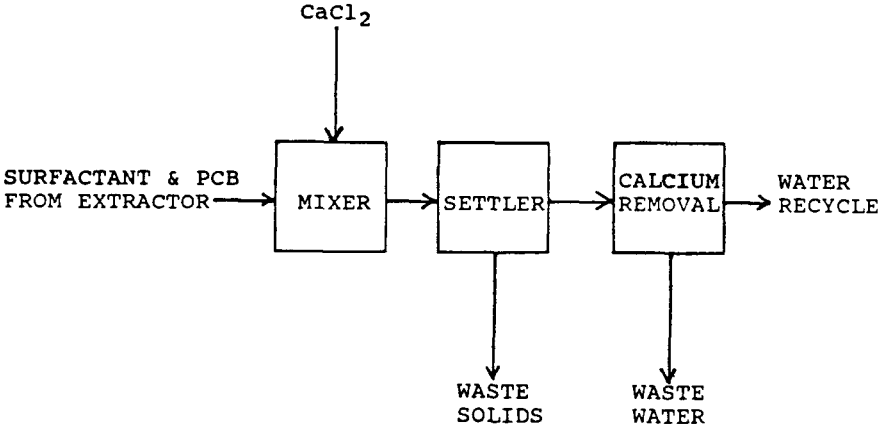


Figure 8-4. Surfactant stream treatment flowsheet.

Any process stream treatment that does not allow the surfactant to be recycled, such as the calcium precipitation method, will have major costs associated with the surfactant loss and the waste disposal. As an example, if 2% SDBS is used with a solid/liquid mass ratio of 1:4, the surfactant cost per cubic yard of soil would be approximately $200 and the cost of waste incineration approximately $400. However, data suggest that the surfactant is underutilized and could therefore be reduced to 1% without adversely affecting the process. This would cut the above costs by half.

SUMMARY AND CONCLUSIONS

The subfill soil at the Oakland site is predominantly a montmorillonite clay. Triton X-100 (nonionic) adsorbs onto montmorillonite clays to a much greater extent than Surco 233 (anionic). As a result of this adsorption difference, Surco 233 outperformed Triton X-100 in the extraction experiments. Subsequently, we will focus on Surco 233 or a similar anionic surfactant for this process.

Stage calculations show that contaminated soil (1000 ppm Aroclor 1260) can be cleaned up to single-digit ppm range in relatively few equilibrium stages. The extraction rate for a stage is moderate with an 80% approach to equilibrium after 20 min.

Due to the nature of clays, solid/liquid separation is difficult, but possible with the use of either large-area thickeners or centrifuges.

Some of the alternatives for process stream treatment are selective adsorption, liquid/liquid extraction, biodegradation, and calcium precipitation. Calcium precipitation is the most promising alternative at this time.

FUTURE PLANS

The major technical challenges that we will address in the coming year are the improvement of solid/liquid separation and the further development of the calcium precipitation treatment of the PCB/surfactant process stream. If other treatment methods are identified that will allow recycle of the surfactant, they will be pursued. Another issue that will be addressed is the potential influence of other soil contaminants, such as mineral oil, on the extraction process. A small bench-scale soil cleanup process will be assembled and operated to elucidate the interactions of the various process variables. This will provide a better technical and economic basis upon which to evaluate the overall process.

Chapter 9

REMOVAL OF POLYCHLORINATED BIPHENYLS FROM AQUEOUS SOLUTIONS: SOLVENT SUBLATION

Sandra L. Woods and Li Tang
Department of Civil Engineering
Oregon State University
Corvallis, Oregon

INTRODUCTION

Polychlorinated biphenyls can be successfully removed from soils by washing the soil with a surfactant. This results in a wastewater containing PCBs, the surfactant, and contaminants from the soil. The purpose of this research project is to develop a simple, effective method to remove PCBs from wastewaters such that the PCBs and the wastewater can be disposed of properly and economically.

Solvent sublation is a simple separation process that can be used to separate dilute solutes from aqueous solutions. This adsorptive bubble separation technique takes advantage of the surface activity and volatility of a solute as driving forces for the removal of the solute from solution. A substance that is not highly volatile or surface active can be removed from solution by the addition of a surface active "collector." Thus the solute can form a complex with the surfactant collector and be removed from solution with the surfactant.

Solvent sublation is a mass transfer process in which a solute is transferred from an aqueous to a solvent phase by gas bubbles. Clean gas bubbles are introduced at the bottom of a column of water containing the solute. In the liquid phase, the trace organic compound in solution may partition into the bubbles, and be removed from the aqueous solution by air stripping, or the solute may accumulate at the gas/liquid interface because of its surface active properties. If additional surfactants are present or are introduced into the solution, the solute may also associate with surfactant molecules or micelles. Introduction of an appropriate surfactant at the proper concentration can encourage the accumulation of the surfactant/solute complex at the gas/liquid interface, and may greatly increase the rate and extent of solute removal from the aqueous solution. Once the bubble has passed into the solvent phase, the solute may dissolve into the solvent or desorb from the surface of the bubble. Surfactant/solute complexes would also re-equilibrate with the solvent.

The objective of the sublation process is to enhance the driving forces that encourage the transfer of trace solutes from the liquid phase to the gas bubbles and from the gas bubbles to the floating solvent phase. Thus, physical characteristics of the process such as bubble size and air flow rate, water column depth, and solvent type and depth would be expected to affect the rate of the process. Characteristics

of the solute such as surface activity, volatility, and affinity for the surfactant would also affect its removal from solution and its retention in the solvent layer.

Specific objectives of this project are

1. To develop a fundamental mathematical model to characterize the mass transfer of PCBs and surfactants during the sublation process
2. To obtain appropriate kinetic and equilibrium constants to describe the transport processes involved in the sublation of PCBs
3. To evaluate the effect of various solvent/surfactant systems on PCB removal.

RESULTS AND DISCUSSION

A fundamental mathematical model to describe mass transfer during the solvent sublation process is being developed while experiments are being performed to increase our understanding of the process. A solution containing 2,4,5-trichlorobiphenyl (2,4,5-CB) and a nonionic surfactant (Triton X-100) has been used in the initial experiments. A glass and teflon column (2.5 cm diameter) containing 240 ml water and 20 ml ethyl acetate solvent is used for the sublation. Air bubbles are introduced at the bottom of the column through fritted glass diffusers.

Mass balances are performed on the chlorobiphenyl with time by measuring concentrations of 2,4,5-CB in the water and solvent phases. Results of an experiment with 0.1-cm bubbles supplied at an air flow rate of 100 ml/min are shown in Figure 9-1. A total mass of 0.035 mg 2,4,5-CB was added to the aqueous phase (240 ml). The initial surfactant concentration was 21 mg/l. After 6 min of sublation, approximately 30% of the PCB remained in the liquid phase and 20% appeared in the solvent. The remaining mass could not be accounted for by the mass balance. After 20 min of sublation, only 0.4% of the PCB remained in the water phase; 95% was measured in the solvent phase; and approximately 3% was not recovered. The mass of PCB that could not be accounted for decreased with time, and it is hypothesized that this mass sorbed to the walls of the sublation vessel at the beginning of the experiment and desorbed with time as the PCBs were removed from the aqueous solution. Measurements of PCB concentrations in a solvent trap on the effluent gas indicated that there was no loss of PCBs from the reactor through the effluent gas.

A similar experiment was performed with 2,4,5-CB in the absence of the surfactant (Figure 9-2). The gas flow rate was held at 50 ml/min and the bubble size was 0.02 to 0.05 cm. This experiment indicated that 2,4,5-CB is also effectively removed from solution due to its own volatility and hydrophobicity. After 20 min, less than 1% of the PCB remained in the liquid phase.

SUMMARY AND CONCLUSIONS

Solvent sublation is a simple separation process that can remove PCBs from aqueous solution and concentrate them in a floating solvent. With gas flow rates

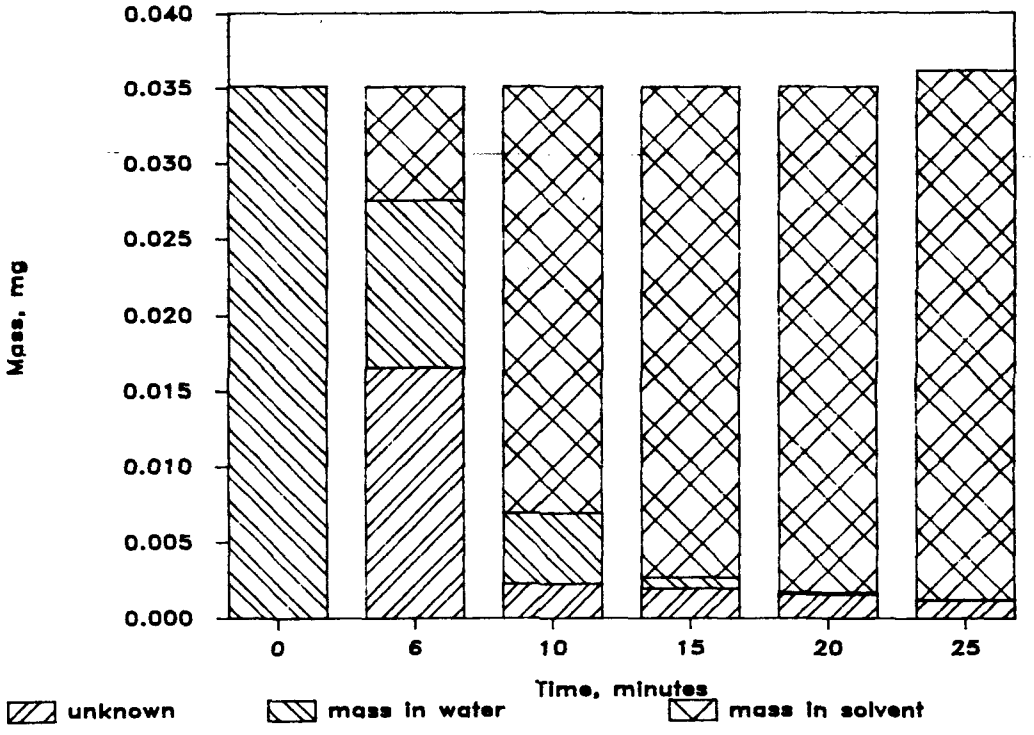


Figure 9-1. **The effect of sublation time on the distribution of 2,4,5-trichlorobiphenyl between water and ethyl acetate in the presence of Triton X-100 (gas flow rate: 100 ml/min; bubble radius: 0.1 cm).**

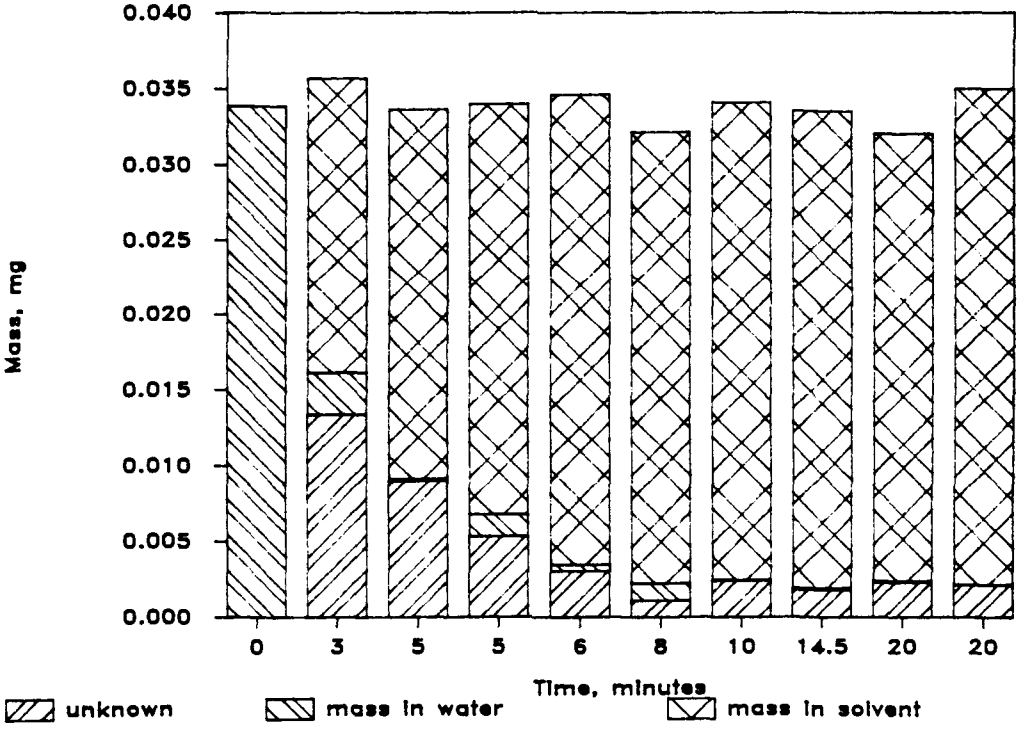


Figure 9-2. **The effect of sublation time on the distribution of 2,4,5-trichlorobiphenyl between water and ethyl acetate without the presence of a surfactant (gas flow rate: 50 ml/min; bubble radius: 0.02-0.05 cm).**

between 50 and 100 ml/min and bubble sizes between 0.02 and 0.1 cm, sublation for less than 1/2 h results in less than 1% of the PCB remaining in the liquid phase. Additionally, transfer of PCB occurs whether or not a surfactant is present.

FUTURE PLANS

Removal of Polychlorinated Biphenyls from Aqueous Solution: Solvent Sublation

Studies of solvent sublation for the removal of PCBs will continue with synthetically contaminated wastewaters. A mathematical model incorporating characteristics of the process, the PCB, and the surfactant will be completed and verified. Once the process has been modeled, additional studies will be conducted to optimize the solvent sublation process for PCBs and to evaluate the removal of PCBs from wastewater generated by washing soils contaminated with PCBs.

Removal of Polychlorinated Biphenyls from Aqueous Solution: Anaerobic Degradation of PCBs in a Continuous Flow Reactor

The purpose of this project is to develop a continuous biological wastewater treatment process to treat wastewaters containing chlorinated biphenyls. Two reactor designs have been used: an upflow anaerobic sludge blanket and a submerged anaerobic filter. Both reactors have been seeded with a mixture containing anaerobic Hudson River sediment from PCB "hot spots" and a consortium of bacteria capable of dechlorinating aromatics. In both reactor configurations, wastewater containing PCBs, nutrients, and alternative carbon sources is pumped into the bottom of the reactor and flows out the top of the reactor. In the sludge blanket reactor, bacteria settle into a blanket at the bottom of the reactor. In the anaerobic filter, glass rings are used as a media for bacterial attachment. In both reactors the objective is to pass wastewater through the system while retaining the bacteria in the reactor. The anaerobic filter is expected to retain more "acclimated" microorganisms, thus increasing the rate of PCB biodegradation. One anaerobic filter is operating now, and several replicates of this anaerobic filter will be constructed this summer so that a variety of environmental conditions (carbon sources, pH, temperature) can be tested.

In these reactors, many surfaces (bacteria, reactor vessel, glass bacterial support rings) are available for sorption of PCBs. A simple mass balance on the influent PCBs would not be sufficient to confirm biodegradation because of loss of these compounds in the reactor or analytical errors (which may be less than 5%). Thus it is difficult to confirm biodegradation without the appearance of metabolites.

Throughout the past year, the effluent from the anaerobic upflow sludge reactor has been monitored for the appearance of dechlorinated PCBs or other PCB metabolites. Although metabolites have not yet been observed, this does not preclude PCB biodegradation. In these continuous flow tests, PCB degradation must be verified by the appearance of metabolites, which must accumulate to a level that can be measured by our analytical techniques. However, the kinetics of the degradation

reactions may prevent the accumulation of metabolites. If the rate of degradation of the metabolite is faster than that of the parent compound, metabolites will accumulate to measurable concentrations only within a limited set of retention times. If the retention time of wastewater in the reactor is too short, degradation of the parent compound will not be sufficient to observe metabolites. If the retention time in the reactor is too long, the metabolites may be degraded below detection level. Thus studies will continue this summer with several anaerobic filters in the hope of increasing the rate of the degradation of the parent compounds. By selection of appropriate environmental conditions and retention times, it is expected that PCB degradation will be observed in continuous anaerobic treatment of PCBs.

RESEARCH PLANS FOR 1987-1988

Our research for the next year will emphasize five areas: (1) the genetics and biochemistry of the bacterial oxidation of PCBs, (2) biologically mediated reductive dechlorination of PCBs, (3) further identification of PCB transformations in the environment, (4) development of a process for the biodegradation of PCBs on soil, and (5) further development of laboratory scale models for surfactant extraction of PCBs from soil.

The genetics and biochemistry of bacterial oxidation of PCBs. The major planned biochemical research efforts will be (1) to isolate and characterize the enzymes of the PCB/biphenyl degradation pathway, and (2) to use mutants blocked at various stages of the PCB/biphenyl degradation pathway to accumulate PCB metabolites for isolation and identification. Various aspects of this work will be conducted in Dr. Gibson's laboratory at the University of Texas at Austin, and at GE.

Subcloning studies designed to minimize the size of the DNA fragment(s) encoding the genes of the PCB/biphenyl degradative pathway will be continued at GE. These will facilitate in mapping the locations of the genes and in obtaining their DNA sequences. From the DNA sequences it will be possible to determine the structure and location of the regulatory regions that control expression of the PCB degradative genes and the amino acid sequences of the enzymes responsible for PCB degradation. This information will provide a basis for the construction of recombinant strains with higher levels of PCB-degradative enzymes.

Reductive dechlorination of PCBs. Drs. Tiedje, Quensen, and Boyd at Michigan State University will continue their laboratory studies of PCB dechlorination using anaerobic batch cultures. Specific objectives of their research include (1) evaluation of the PCB-dechlorinating activity of sediments from other PCB-contaminated sites, (2) evaluation of the dechlorination of other congeners, especially those of greater toxiciological importance, such as 3,4,3',4'-CB, and (3) enhancement of the rate of dechlorination by the addition of nutrients or solubilizers.

Dr. Woods and her colleagues at Oregon State University will continue their evaluation of anaerobic PCB degradation in two different kinds of continuous flow reactors. Several replicates of an upflow anerobic filter reactor will be constructed so that PCB degradation can be evaluated under various conditions (carbon sources, pH, and temperature will all be varied).

At GE we will investigate the possibility of using the hydrogenases of aerobic bacteria to dechlorinate PCBs. These enzymes contain nickel at the active site and are able to reduce a broad range of substrates. They are good candidates for aerobic PCB dechlorinases because it has been shown that hydrided (Raney) nickel and nickel-hydrogen combinations can catalyze the hydrodechlorination of PCBs.

Environmental transformations of PCBs. We will continue our studies of reductive dechlorination processes that occur in anaerobic sediments and the environmental factors that allow them to proceed. We will also investigate the aerobic biodegradation of PCBs in natural waters and possible routes to its acceleration.

Biodegradation of PCBs in soil. The major focus of this work will be a field test at a drag strip contaminated with Aroclor 1242. The test will involve repeated applications of *Pseudomonas putida* LB400. In the laboratory we will continue to optimize conditions for biodegradation of PCBs on soil and will determine the feasibility of using mixed cultures of LB400 and *Corynebacterium* sp. MB1.

Surfactant extraction of PCBs from soil. In this area we will evaluate possible solutions to two critical technical issues: (1) the separation of PCB-surfactant solutions from the soil, and (2) the removal of PCB from surfactant wash solutions. In the first area, we are attempting to improve separation through the use of flocculating and coagulation agents. In the second area, the most promising method at present is calcium precipitation of the PCB-laden surfactant to allow recycling of the water. We are continuing our evaluation of this process at GE.

At Oregon State University, Dr. Woods and her colleagues will continue to study solvent sublation of PCBs. Initially these studies will be conducted with defined solutions of surfactant and PCB. A mathematical model incorporating characteristics of the process will be completed and verified. Once the process has been modeled, additional laboratory studies will be conducted to optimize the solvent sublation process for PCBs, and to evaluate the utility of this process for the removal of PCBs from wastewater generated by washing PCB-contaminated soils.

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